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Clinico-microbiological profile of healthcare associated infections in a neonatal intensive care unit and its relation with environmental surveillance

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ABSTRACT

Introduction: Healthcare associated infections (HAI's) or nosocomial infections are frequently encountered in neonatal intensive care units (NICU's), resulting in morbidity as well as mortality of new born babies, with varying predisposing factors and microbiological profile.
Objective: This study was conducted to identify the most common HAI among the neonates admitted in the NICU, to identify the risk factors of HAI's in neonates and to compare the bacteriological profile of culture positive cases with the results of environmental survey.
Materials and Methods: This was a prospective study, conducted for a duration of one and a half years from December 2018 to May 2020. Environmental samples from the NICU as well as clinical samples from neonates admitted to the NICU of a tertiary care centre for a duration of more than 2 calendar days were collected.
Results: The most common HAI in the NICU was VAP (41.6%), followed by CLABSI (21.8%). Statistically significant risk factors for neonatal nosocomial infection were preterm birth, LSCS, presence of comorbidities, Low Birth Weight, absence of breast feeding and Apgar score. The most common Gram negative organism isolated was Acinetobacter baumannii (18.8%) and Gram positive organism as Methicillin Resistant Coagulase negative Staphylococcus (MRCoNS) (13.9%).
Acinetobacter baumanni above average isolation rate was matching with the growth of the similar organism each month, according to the surveillance report.
Conclusion: The most common HAI among neonates in our study was VAP, majority of which were caused by Gram negative bacteria. Acinetobacter baumanni was found to have an isolation rate above average on environmental surveillance, indicating its role in causing HAI's in neonates admitted to the NICU. However, this study did not show a definitive association between HAI and the environmental surveillance report.

1. Introduction

Healthcare Associated Infections (HAI's), also called nosocomial infections (NI) is defined as a systemic or localized condition that was not incubating at the time of hospital admission, due to adverse reaction to an infectious agent(s) or its toxin(s).[1] The symptoms should appear after at least 2 calendar days admission to the hospital for a cause other than the infection in the background.[2] The HAI rate indicates the safety of care and quality of a facility, and hence monitoring HAI rates is considered as a key factor required for secure and high-quality healthcare, particularly in intensive care units (ICU's).[3] The incidence of nosocomial infections is reported to show a large variance with neonatal sepsis from India, ranging from 1.5 to 37%, which is 3 to 20 times greater than in developed

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countries.[3]

HAI's in neonates are late-onset in nature, or infection after the first 2 calendar days of birth,[1] with exceptions of Group B Streptococcal or Herpes simplex virus infection.[4] The most commonly encountered HAI's are central line associated blood stream infection (CLABSI), ventilator associated pneumonia (VAP), catheter associated urinary tract infection (CAUTI) and surgical site infections (SSI's).[5] The other possible HAI's include meningitis, conjunctivitis and skin infections.[6]

Colonization of the CVC with bacteria and infusate contamination are the main causes of CLABSI, the origin of which may be either extra luminal or intraluminal.[7] VAP is the 2^{nd} most common HAI, and accounts for 6.8% to 32.3% of NICU HAI's.[8] It's a major cause of morbidity as well as mortality in critically ill patients who are on invasive mechanical ventilation (MV) through an endotracheal tube (ETT) or via tracheostomy.[9] CAUTI accounts for upto 40% of all HAI's world wide, probably because urinary catheterisation is required in 15-25% of hospitalised patients.[2] SSI refers to development of an infection at an incision site after a surgical procedure. According to reports, SSI rate in India ranges from 4.1 to 11 per 100 surgeries.[2]

Microbial surveillance, improved infection control initiatives and enhanced local skin care have decreased the rate of colonization and diseases caused by Group I organisms like Staphylococcus aureus.[6] Klebsiella species is emerging threat to the sick, frail and debilitated newborns.[10] Approximately 40% of Escherichia coli strains that cause septicemia have K1 capsular antigen.[11]

This study aims to evaluate the most common HAI's, risk factors and microbiological profile of neonatal HAI's in the NICU and correlate it with the environmental surveillance.

2. Materials and Methods

This observational, descriptive and prospective study was carried out in the department of Microbiology. 101 samples from neonates in the NICU with signs & symptoms of infection and the environmental surveillance samples from the NICU of the tertiary care centre. The study duration was for one and a half years from December 2018 to May 2020. All neonates admitted to the NICU for a duration more than 2 calendar days, and suspected to have developed a HAI were included in the study. The neonates presenting with signs & symptoms of infection less than 2 calendar days, along with those who were discharged home & later presented with sepsis were excluded from the study.

All basic demographic details of mother and neonate risk factors for an HAI such as, low birth weight, gestational age, gender, mode of delivery, Apgar score at 1 and 5 min less than 7 and co-morbidities if any were noted. Data on invasive device usage (central / umbilical arterial / venous lines, urinary catheters and ventilator), duration of device usage and duration of hospital stay were also collected.

The study group neonates were categorized into site specific HAI's using Centre for Disease Control and Prevention proposed standard defintions.[12] The required samples (blood, cerebrospinal fluid, urine, endotracheal tube aspirates etc.) were collected under aseptic precautions, processed according to standard methods, and if any organism was present it was further processed and identified using standard microbiological techniques.[13]

Blood samples were analyzed using sepsis screen (Absolute neutrophil count, C- reactive protein, Peripheral smear for sepsis) as well as blood culture. According to CDC guidelines,[12] 1–2 ml of blood was taken both from the central and peripheral lines, into Bactec bottle and kept for incubation in the Bactec 9120 instrument. CLABSI was diagnosed by calculating the differential time to positivity of the growth obtained. Cerebrospinal spinal fluid (CSF) samples were also examined for cell count and protein & glucose estimation, in addition to Gram's stain, culture and biochemical tests to identify the bacteria. 1–5 drops of CSF was inoculated onto the culture media and incubated for a period of 24 hrs. Any bacterial growth if present was further identified and subjected for antibiotic sensitivity testing. Supra pubic aspiration or catheterization technique was used for urine sample collection, following which routine microscopic examination, culture & sensitivity were done. In the applicable study subjects, endotracheal tube, ear discharges and conjunctival secretions were also collected for culture and sensitivity.

Data was also collected of the total number of babies on devices and the total number of device days / surgeries, from the NICU. The HAI incidence rates of device / surgery associated HAI's were calculated for each month during the study period using the formula:[12]

VAP / CLABSI / CAUTI rate calculated as = $\frac{Total\ number\ of\ infections\ X\ 1000}{Total\ number\ of\ device\ days}$

SSI rate calculations = $\frac{Total\ number\ of\ infections\ X\ 100}{Total\ number\ of\ surgeries\ performed}$

Every 4 weeks prior to fumigation, regular environmental surveillance was carried out in the NICU. Environmental surfaces that were regularly screened in the NICU included: phototherapy lights, radiant warmers, baby cot, weighing machine, suction tubings, laryngoscopes, feeding trays, thermometers, feeding counter, air etc. Sterile cotton-tipped swabs were used to sample the surfaces of inanimate articles, by moistening the swabs with sterile phosphate buffered saline and then it was streaked across a 4-in^2area. At the same time, culture plates were exposed to room environment of both the NICUs for one hour for air sampling using an air sampler. Water samples were collected from ventilator humidifiers for water culture and processed. The swabs were inoculated on nutrient agar and were incubated at 37°C under aerobic condition for 18-24 hours. The NICU staff were advised to get the surface

disinfection & fumigation done as per protocol, in case of a significant growth from any environmental sample. Inorder to ensure decontamination, subsequent sampling was done from the contaminated article after proper disinfection measures. If the increase in the isolation percentage during a specified period was matching with the monthly growth of that organism in the surveillance, it was presumed to have some association. The sample size 'n' was calculated according to a study by F. Abdel Wahab in Egypt, where the prevalence of HAI's in the NICU was 21.4%.[13] n = (Z_(∝^2) " p (1-p) ")/e^2 = 101, at e = 8%

Statistical analysis was performed using the SPSS program for Windows, version 25.0. The data was presented as frequency percentages. Chi square test was used to calculate the p value and analyse the association between different variables & neonatal sepsis, as well as that with environmental surveillance.

3. Results

The general characteristics of the study group were analysed (Table 1). Samples cultered include blood, ET tip, urine, Wound swab, CVC tip and CSF (Table 2). Majority of the organisms isolated (53.4%) were Gram Negative bacteria, the most common, i.e 19 (18.8%) being Acinetobacter baumannii. Among the 44 (43.5%) Gram Positive organisms isolated, the most common, i.e 14 (13.9%) were Methicillin Resistant Coagulase Negative Staphylococcus. Remaining 3 (2.9%) were Candida albicans (Figures 1, 2 and 3). The most common HAI was VAP (41.6%) (Figure 4). The incidence rates of HAI's in each month was as shown in Figure 5 and statistically significant risk factors for these HAI's are shown in Table 3.

3.1. Environmental surveillance

The percentage of isolation of the predominant pathogen Acinetobacter baumannii in the NICU culture reports were noted each month for the study period, and its average was calculated (Table 4). The average isolation rate of Acinetobacter baumannii in the NICU was 18.8%. The month of January 2019 had an isolation rate of 21.05%, which exceeded average isolation rate. February 2019 had an isolation rates of 26.3% but the surveillance report showed no growth. In the months of June and August 2019, surveillance report showed growth but isolation rates were below average isolation rates, i.e 5.2% and 0% respectively. However, the sensitivity of isolates of environmental samples did not match with that of the clinical isolates, indicating they are of different strains. Some vague association is shown between HAI and environmental surveillance report, but its significance is yet to be known.

Table 1: General characteristics of the study group

Characteristic	Percentage (No.)
Gender:	
Male	74.3 (75)
Female	25.7 (26)
Place of birth:	
Inborn	72.3 (73)
Outborn	27.7 (28)
Gestational age:	
Term	43.6 (44)
Preterm	56.4 (57)
Apgar score at 1 minute:	
Unknown	22.8 (23)
0 to 3	2 (2)
4 to 6	18.8 (19)
7 to 10	56.4 (57)
Apgar score at 5 minutes:	
Unknown	22.8 (23)
0 to 3	0 (0)
4 to 6	4 (4)
7 to 10	73.3 (74)
Mode of delivery:	
Vaginal	34.7 (35)
LSCS	65.3 (66)
History of feed aspiration:	
Yes	9.9 (10)
No	90.9 (91)
Superficial infection:	
Yes	13.9 (14)
No	86.1 (87)
Comorbidities:	
Yes	79.2 (80)
No	20.8 (21)
IV fluids:	
Yes	95(96)
No	5 (5)
Breast feeding	
Yes	30.7 (31)
No	69.3 (70)
Invasive device	66.3 (67)
Surgery	7.9 (8)

Table 2: Sample cultured

Sample	Percentage (No.)
Blood	60.4 (61)
ET tip	11.9 (12)
Urine	5.9 (6)
Wound swab	9.9 (10)
CVC tip	7.9 (8)
CSF	1 (1)

Table 3: Statistically significant risk factors

VAP	CLABSI	CAUTI	SSI
Preterm birth LSCS Absence of breast feeding Apgar score	Presence of comorbidities Apgar score	None	Preterm birth LSCS Low birth weight

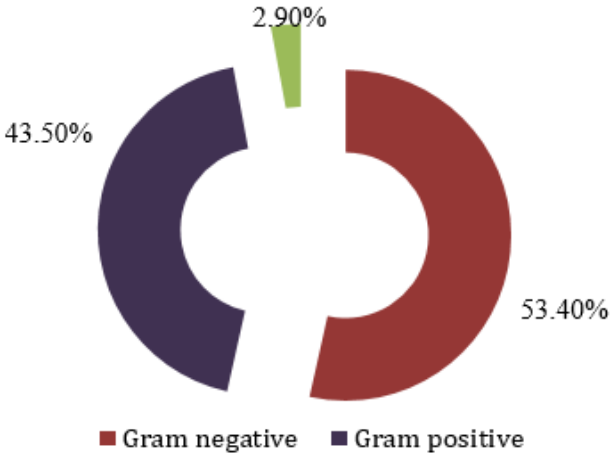

Fig. 1: Organism isolated

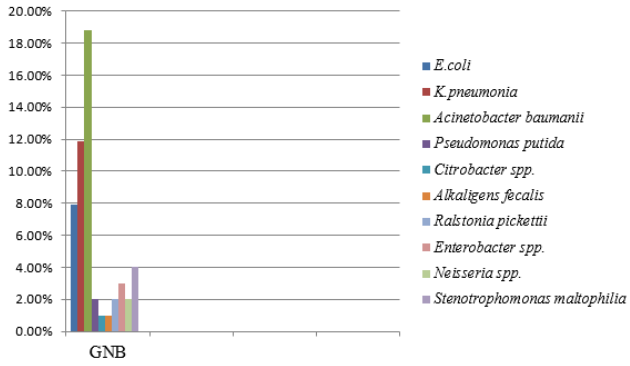

Fig. 2: Gram Negative bacteria isolated

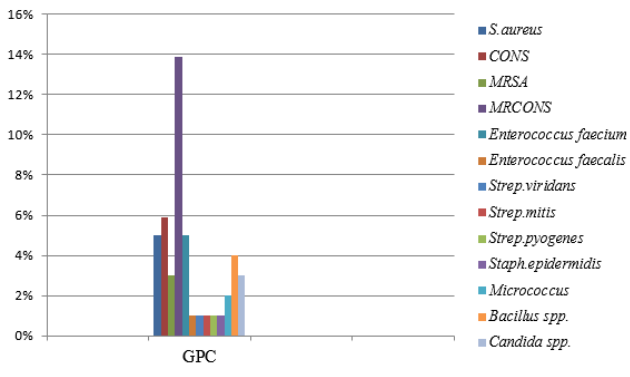

Fig. 3: Gram Positive bacteria and Fungus isolated

Table 4: Environmental surveillance report from the NICU

Month	Growth
December 2018	No growth
January 2019	Ventilator – CONS Floor – CONS Infusion pump – CONS Suction tubing – Acinetobacter baumanni Oxygen humidifier – Acinetobacter baumanni
February 2019	No growth
March 2019	No growth
April 2019	Ventilator – MRCONS Infusion pump – Staphylococcus aureus Stethoscope – CONS
May 2019	Ventilator – CONS
June 2019	Suction pump – Acinetobacter baumanni
July 2019	No growth
August 2019	Suction pump – Acinetobacter baumanni
September 2019	No growth
October 2019	No growth
November 2019	Oxygen humidifier – CONS
December 2019	Ventilator – MRCONS
January 2020	No growth
February 2020	Stethoscope – CONS
March 2020	Infusion pump – Staphylococcus aureus
April 2020	No growth
May 2020	No growth

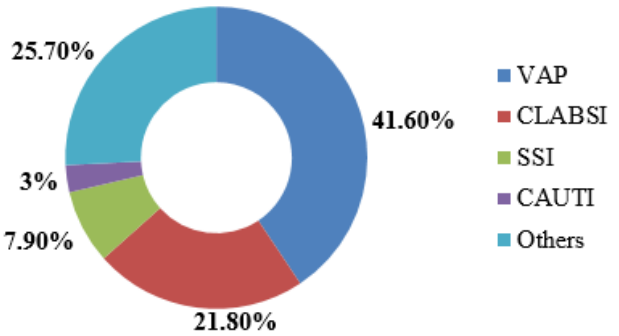

Fig. 4: Percentage of HAI's

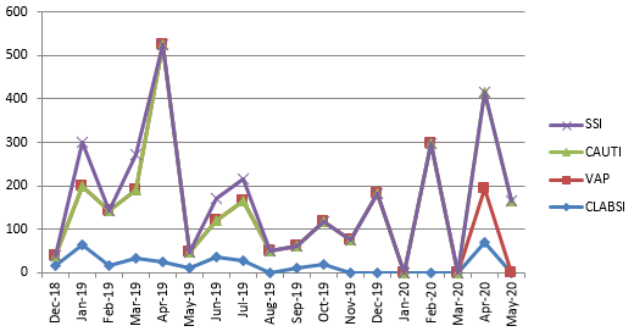

Fig. 5: Incidence rates of HAI's

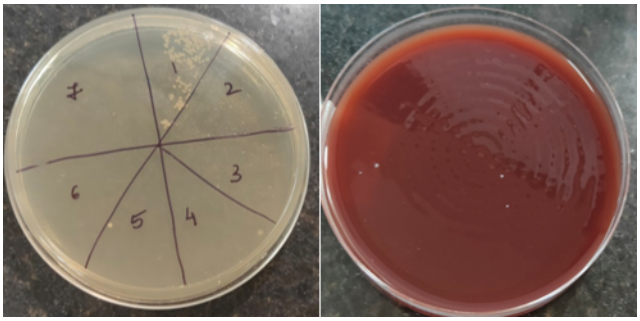

Fig. 6: Environmental samples cultured on nutrient agar and bloodagar

4. Discussion

HAI's continues to pose serious threat to neonates, and many advances have been made in neonatal care that have resulted in an increased chances of survival of smaller and weaker babies.[6] These infections are associated with complications like increased mortality rates, immediate and long term morbidity, prolonged hospital stay and increased cost of care.[14,15] Hand hygiene, use of maternal breast milk and strict adherence to aseptic protocols in neonatal NICU's are of utmost importance inorder to keep the infection rates low.[16]

The most common HAI among neonates found in our study was VAP (41.6%), followed by CLABSI. This was in concordance with the study by F. Abdel Wahab et al.[17] However, in a study by Kumar S et al,[1] CLABSI (73%) was the most common HAI, followed by VAP (12%).

Overall, the statistically significant risk factors for HAI in our study were found to be preterm birth, LSCS, presence of other co-morbidities, low birth weight, absence of breast feeding and an Apgar score at 5' less than 7. This was similar to studies by Kumar S et al.[1] However, Jeong et al[18] and Prado DS et al[19] demonstrated that there was no association between HAI's and above mentioned factors.

Gram negative bacteria were predominant (53.4%) in our study, the most common organism being Acinetobacter baumannii, followed by Klebsiella pneumonia, F. Abdel Wahab et al[17] found that Klebsiella spp. were the most frequently isolated organisms followed by E. coli, Citrobacter koseri, Pseudomonas putida, Stenotrophomonas maltophilia, Ralstonia pickettii and Alkaligens fecalis similar to the results of previous Egyptian studies.[20,21] The identification of gram positive organisms in cultures from patients can be difficult due to the differentiation between colonization and infection, for example, CoNS are typically found on the newborns skin.[22] Bacillus spp was isolated from 4% babies with VAP, along with Enterococci, Micrococcus spp and Streptococcus mitis, 85.1% of the babies with HAI recovered with appropriate treatment and were discharged home. Antibiotics were started on the basis of resistance patterns of the organisms isolated and antibiotic susceptibility reports done by Kirby Bauer disc diffusion method and CLSI guidelines.[23] Other supportive care including IV fluids, caffeine, vitamin K, total parenteral nutrition, breast milk or paladai feeds, and symptomatic therapy was also given to the neonates. NICU surfaces harbour microbial ecosystems, influenced by contact with their residents, i.e innately vulnerable premature and sick neonates.[24]

In our study, the above average isolation rate of Acinetobacter baumanni was matching with the monthly growth of a similar organism according to the surveillance report. In the study by Kumar S et al[1] S. aureus was the dominant pathogen isolated from neonates as well as the environment. This correlation between organisms from the HAI and environmental surveillance may not show cause-effect relationship. Though environmental cultures may be one of the predicting causative factors that contribute to late onset sepsis, its statistical significance is yet to be proven. Molecular typing, along with studies on survival period of organisms on different inanimate articles and restricting collection of blood samples for culture to that period may be helpful in confirming the association between HAI and environmental surveillance. We still lack knowledge regarding answers to such queries which require more elaborate studies.

In order to prevent nosocomial infections, NICU's must strictly follow infection control bundles and routine bedside cleaning and disinfection of nursery equipment, and there are studies that demonstrate a reduction in bacterial colonization after routine cleaning practices.[25] Based on our study results, NICU staff was advised to conduct surface disinfection & fumigation using 5% Silvicide (10% Hydrogen peroxide + 0.01% Silver nitrate) 20% Silvicide respectively, following which subsequent sampling was done from the contaminated articles to ensure decontamination. However, the effect of routine cleaning on colonization by commensal and pathogenic bacteria in infants is still unclear.

Suction pump and oxygen humidifier were the main environmental sources of infection in our study. Humidified incubators, being beneficial for maintaining the skin integrity and temperature regulation of neonates, are a mainstay equipment in treating preterm infants in NICU's. But the microbes can also grow in warm moist environment, especially Staphylococci.[25] Even after all this, the frequency with which incubators are to be cleaned inorder to minimize the risk of infections, while maintaining infant safety is unknown.

5. Conclusion

Hospital acquired infection (HAI) in neonates is one of the most common complications posing serious health hazard as it leads to increased morbidity, mortality, duration and costs associated with hospital stay. The most common

HAI among neonates was Ventilator Associated Pneumonia, followed by Central Line Associated Bloodstream Infection. Gram negative organisms were responsible for majority of the infections, among which Acinetobacter baumannii and Klebsiella pneumoniae were the most common. The significant risk factors associated with HAI were found to be preterm birth, lower segment caesarean section, presence of other co-morbidities, low birth weight, absence of breast feeding and an Apgar score at 5' less than 7.

On environmental surveillance, Acinetobacter baumanni was found to have an isolation rate above average, indicating its possible role in causing HAI in neonates admitted to the NICU. But this study shows only a vague association of HAI with the environmental surveillance report and its significance is yet to be known. This shows a need for multicentric neonatal nosocomial infection surveillance systems with standardised definitions and reporting formats, inorder to generate information on neonatal HAI rates and predisposing factors in our setting, using which appropriate preventive strategies can be taken up.

6. Conflict of Interest

None.

7. Source of Funding

None.

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