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Article

Incidence and Determinants of Health Care Associated Blood Stream Infection at a Neonatal Intensive Care Unit in Ujjain, India: A Prospective Cohort Study

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Abstract: Very little is known about healthcare-associated infections (HAIs) in neonatal intensive care units (NICUs) in resource-limited settings including, India. The aim of this prospective study was to determine the prevalence, onset, risk factors and causative agents of laboratory confirmed blood stream (LBCI) as a HAI in a level-2 NICU at RD Gardi Medical College, Ujjain, India. The diagnosis of HAI was established using the Centre for Disease Control, USA criteria. A predesigned questionnaire containing risk factors associated with BSHAI was filled. A total of 150 neonates (43% preterm) were included in the study. The incidence of LBCI was 31%; 56% of which was late onset sepsis. The independent risk factors for LBCI were: preterm (Odds Ratio OR 3.22), duration of NICU stay more than 14 days (OR 2.38), chorioamnionitis in the mother (OR 18.48), neonate born through meconium stained amniotic fluid (OR 4.53), mal-presentation (OR 10.58), endotracheal intubation (OR 11.60), umbilical catheterization (OR 15.11), HAI due to ventilator-associated pneumonia (VAP) (OR 11.88). Initiation of minimal enteral nutrition was protective (OR 0.15). The predominant causative organisms were Gram-negative pathogens (58%). Among *Klebsiella* spp. and *E. coli* isolates, 73 and 80%, respectively were identified as extended-spectrum beta-lactamase producers. The results can be used to identify high-risk neonates for LBCI.

Keywords: blood stream health care associated infections; neonates; risk factors, antibiotic use, antibiotic resistance; neonatal intensive care unit; India

1. Introduction

Health care associated infections (HAI) are a significant public health problem resulting in increased morbidity, mortality and increased length of hospital stay and health care costs [1]. Neonates admitted to Neonatal Intensive Care Units (NICU) are more vulnerable to HAI because of their underlying susceptibility to infection and the need for invasive procedures [2].

Various fetal, maternal and NICUs environmental factors contribute towards causing infections in newborns. Some of the fetal factors apart from the immature immune system, are low birth weight, gestational age and Apgar score, prolonged hospital stay, invasive procedures, endotracheal tubes, umbilical cauterization, parenteral nutrition, lack of adequate hand washing by hospital personnel

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and indiscriminate use of antibiotics [1-4]. Some of the maternal factors are premature rupture of membrane, maternal fever within 2 weeks prior to delivery, meconium stained amniotic fluid (MSAF), foul smelling liquor and instrumental delivery etc.[1-4]. NICUs environment can be bacteriologically very hostile, containing a wide selection of pathogenic, antibiotic resistant organisms with which the patient becomes colonized [1-4].

Among the neonatal infections in the NICU, blood stream infections (BSI) are a major cause of concern worldwide [1,4-7]. In low-middle income countries (LMICs) BSI rates are two to three times higher as compared to high-income countries [1,3,4,7,8]. In high-income countries, HAI surveillance in the intensive care unit (ICU) plays a major role in infection control as HAI especially those caused by multi-drug resistant bacteria is essential to report [1,4,6]. However, there is paucity of data from LMICs including India where HAIs continue to remain a hidden but serious burden for health systems and patients alike [1,7,9-11]. Thus the present study was undertaken with the aim to determine the incidence, onset, risk factors and causative agents associated with blood stream HAI in a level-2 NICU at C. R. Gardi hospital (CRGH), Ujjain, Madhya Pradesh, India.

2. Materials and Methods

2.1 Settings

The study was conducted from June 2012 to January 2014 in the eight-bedded NICU, which is a part of 750-bedded academic hospital CRGH, and is associated with R. D. Gardi Medical College, Ujjain. The hospital is situated in a semi – urban area approximately six km from Ujjain city in western part of the province of Madhya Pradesh, India. The hospital caters predominantly to rural population from the villages surrounding Ujjain city. During the study period, three post-graduate trainees and four consultants provided the patient care in NICU. The nurse to patient ratio during the study period ranged from 1:8 to 1:12. The bed occupancy remained above 98% during the study period. Our nursery admits a mixed population of low and high-risk infants. Aseptic non-touch techniques and maximum aseptic precautions were available during the study period, but compliance with these measures was not monitored.

2.2 Definitions

For defining clinical sepsis and laboratory confirmed blood stream infection (LCBI) the standard criteria for HAI surveillance as defined by the Centers for Disease Control and Prevention (CDC's), the National Nosocomial Infection Surveillance System (NNIS) and the National Healthcare Safety Network (NHSN) were used [12]. Primary BSI was defined as: (1) a patient with a recognized pathogen cultured from one or more blood cultures, where the organism cultured was not related to an infection at another site; or (2) a patient found to have a common skin contaminant (e.g., coagulase-negative staphylococci, viridans group streptococci or micrococci, diphtheroids, *Bacillus* sp., *Propionibacterium* sp.) cultured from two or more blood cultures [13]. Additionally, the presence of at least 1 of the signs and symptoms for the diagnosis of infection was necessary, including thermal or hemodynamic instability, apnea, milk or glucose intolerance, respiratory distress, hemodynamic instability or underactivity/lethargy [14]. Ventilator Associated Pneumonia (VAP) was defined as per NNIS definitions [12].

LCBI in neonates admitted in NICU and presenting within the first 72 hours of life were defined as early onset sepsis (EOS) and those presenting after 72 hours of life were defined as late onset sepsis (LOS) [15]. New Ballard scoring was used to calculate gestational age of newborns after birth [16]. The babies were classified as appropriate, small or large for gestational age (SGA or LGA) according to Lubchenco and Battaglia charts [17]. The birth weight of the neonates was categorized by adopting the NNIS, USA for high-risk neonatal intensive care units. The following three birth weight categories: 1001–1500 g, 1501–2500 g, > 2500 g were used [18]. Infants < 2.5 kg at birth were

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considered low-birth-weight infants, including those small for gestational age, infants < 1500 g were considered very low- birth-weight infants, and premature infants were those born before 37 weeks of gestation [19]. Mothers with high risk of chorioamnionitis were defined as having 2 or more of the following: maternal fever, lekocytosis, maternal tachycardia, uterine tenderness or foul smelling amniotic fluid [20]. Premature rupture of membranes (PROM) was defined when there was leaking of membranes for more than 24 hours before the onset of labor [20].

2.3 Inclusion and exclusion criteria

Consecutive neonates admitted in NICU during the study period, having a gestational age more than 28 weeks and diagnosed LCBI using the above definitions were included in the study. As there is an inherent uncertainty in ascribing neonatal infection as "maternally acquired", or "hospital acquired" for the study any infection in the hospital-born neonate was considered potential HAI. Neonates less than 1000 g birth-weight, neonates transferred to the NICU with a diagnosis of sepsis and/or showing features of sepsis on admission were not included. HAI caused by fungal organisms and anaerobic bacteria were also not included.

2.4 HAI Surveillance

A pre-designed questionnaire for the surveillance of LCBI as HAI was filled-in for all the neonates included in the study. At least two physicians and one of the four resident doctors, who were trained in HAI surveillance, followed all neonates daily till discharge or death.

The questionnaire contained a) demographic details of the neonates including name of the neonate and the mother, age of the baby at the time of admission, date and time of birth, and address of the mother and b) the maternal, fetal and environmental risk factors associated with LBCI as HAI. Appropriate samples for septic screen and blood cultures were collected on all neonates with suspected sepsis. Empirical antibiotic therapy was started according to antibiotic guidelines for NICU, if CRP was positive, awaiting culture reports. All neonates were followed-up till discharge or death. The number of patient-days was defined as the total number of days that patients spent in the NICU during a 28-day period.

2.5 Blood culture sampling and processing

Blood cultures were obtained by peripheral puncture. There was no change in blood culture sampling policy during the study period. Within four hours of receipt all the samples were plated on blood agar and MacConkey agar medium (HiMedia Laboratories Pvt, Ltd, Mumbai, India). The growth of bacteria within 48 hours after blood culture was considered clinically significant. Pathogenic bacteria were identified using standard conventional microbiological methods [21].

2.6 Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates. The disc strengths were as recommended by the Clinical and Laboratory Standards Institute (CLSI) at the time of the study [22]. CLSI interpretive criteria for susceptibility and resistance were followed [22]. Extended Spectrum Beta-Latamase (ESBL) were detected phenotypically by the combined disc diffusion method with cefotaxime (30μg) and cefotaxime/clavulanic acid (30/10 μg) and ceftazidime (30μg) and ceftazidime/clavulanic acid (30/10 μg) according to CLSI guidelines [22] and were confirmed using Vitek 2 [23]. Multidrug-resistant isolates were defined as isolates having co-resistance to at least three antibiotic groups [24]. The ethics committee of RD Gardi Medical College approved the study (approval number 114/2010). The cultures were performed without any cost to the patients, and the results were made available to the concerned physician.

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2.7 Statistical Analysis

The data was entered in EpiData Entry (version 3.1) and then transferred to Stata 12.1 (Stata Corp. College Station, Texas, USA) software for statistical analysis. Frequency and percentages are presented for categorical data. Pearson Chi square test with 95% confidence interval (95% CI) was used to calculate odds ratios (OR) for potential risk factors. Crude ORs were calculated from two by two tables. Multi-variable logistic regression model was used to calculate adjusted OR in the final model with laboratory confirmed blood stream infection (LCBI) as outcome variable. The dichotomized independent variables included were meconium stained liquor (yes versus no), umbilical catheterization (yes versus no), endotracheal intubation (yes versus no), malpresentation (yes versus no), a history of premature rupture of membranes with fever in mother (yes versus no), chorioamnionitis (yes versus no) and minimal enteric nutrition (yes versus no). Categorical variables included duration of stay in NICU (less than 7 days versus 7 to 14 days and more than or equal to15 days). A *p* value less than or equal to 0.05 was considered significant.

3. Results

A total of 775 neonates were admitted in NICU during the study period and 150 neonates were suspected to have HAI. These 150 neonates formed the final cohort. Out of them 95 (63%) were male. A total of 65 neonates (43%) were preterm babies according to New Ballard scoring. One-fourth (n=41) babies were small for gestational age (SGA) and the rest were appropriate for gestational age (AGA), there were no large for gestational age babies admitted in the unit during the study period. A total of 15 neonates (10%) babies died during the study period. All deaths were among preterm neonates and clinical sepsis due to HAI was suspected in all.

3.1 HAI rates

The rate for microbiologically confirmed LBCI was 46/150 ie 31% (95% CI 23 to 38). The LBCI differed among the males (n=95) who had LBCI rate of 33% (95% CI 23 to 42) and females (n=55) neonates 28% (95% CI 15 to 39). The rate among preterm neonates (n=85) was 44% (95% CI 32 to 57%) compared to term neonates (n=65), 20% (95% CI 11 to 29%). The LBCI rate for early onset sepsis (n=81) was 25% (95% CI 15 to 34) and that for late onset sepsis (n=69), 38% (95% CI 26 to 49).

3.2 Risk factors for HAI

The following risk factors were significantly associated with microbiologically confirmed LBCI in multivariate analysis: term versus preterm (OR 3.22, 95% CI 1.56 to 6.63; p=0.001), duration of NICU stay more than 14 days versus less than 14 days (OR 2.38, 95% CI 1.08 to 5.22; p=0.03), chorioamnionitis in mother (OR 18.48, 95% CI 2.20 to 155.16; p=0.007), PROM in mother (OR 6.06, 95% CI 2.24 to16.33; p<0.001), neonate born through meconium stained amniotic fluid (MSAF) (OR 4.53, 95% CI 1.53 to 13.38; p=0.006), mal-presentation (OR 10.58, 95% CI 2.78 to 40.13; p=0.001), endotracheal intubation (OR 11.60, 95% CI 3.94 to 34.16; p<0.001), umbilical catheterization (OR 15.11, 95% CI 5.78 to 39.50; p<0.001), HAI due to ventilator-associated pneumonia (VAP) (OR 11.88, 95% CI 3.16 to 44.63; p<0.001). Initiation of minimal enteral nutrition was protective from HAI, (OR 0.15, 95% CI 0.04 to 0.51; p<0.001). The details are shown in Table 1. For each increasing day of stay in NICU the risk for LBCI increased by 6.6% (SE 2.9%). Also, for each incremental day a neonate was on intravenous fluids the risk of LCBI increased by 12% (95% CI 6 to 26%; P=0.039).

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177 Table 1 Multivariate analysis of neonatal and maternal characteristics associated with laboratory
178 confirmed blood stream infections in a cohort of 150 neonates

Risk factors	Total (%)*	HAI		OR	95% CI	*p* value
	150	No (%)#	Yes (%)#			
Sex						
Female	55 (37)	40 (73)	15 (27)	1.29	0.62 – 2.86	0.493
Male	95 (63)	64 (67)	31 (33)			
Gestational age						
Term	85 (57)	68 (80)	17 (20)	3.22	1.56 – 6.63	0.001
Preterm	65 (43)	36 (55)	29 (45)			
Duration of NICU stay						
Up to 14 days	115 (77)	85 (74)	30 (26)	2.38	1.08 – 5.22	0.030
>14 days	35 (23)	19 (54)	16 (46)			
Choroamnionitis in mother						
No	142 (95)	103 (73)	39 (27)	18.48	2.20 – 155.16	0.007
Yes	8 (5)	01 (12)	7 (88)			
PROM in mother						
No	129 (86)	97 (75)	32 (25)	6.06	2.24 – 16.33	<0.001
Yes	21 (14)	7 (33)	14 (67)			
Meconium stained amniotic fluid						
No	134 (89)	98 (73)	36 (27)	4.53	1.53 – 13.38	0.006
Yes	16 (11)	6 (37)	10 (13)			
Malpresentation						
No	136 (91)	101 (74)	35 (26)	10.58	2.78 – 40.13	0.001
Yes	14 (9)	3 (21)	11 (79)			
Birth asphyxia						
No	122 (81)	86 (70)	36 (30)	1.32	0.55 – 3.15	0.522
Yes	28 (19)	18 (64)	10 (36)			
ET intubation						
No	128 (85)	99 (77)	29 (23)	11.60	3.94 – 34.16	<0.001
Yes	22 (15)	5 (23)	17 (77)			
Umbilical cannulation						
No	119 (79)	97 (82)	22 (18)	15.11	5.78 – 39.51	<0.001
Yes	31 (31)	7 (23)	24 (77)			
VAP						
No	135 (90)	101 (75)	34 (25)	11.88	3.16 – 44.63	<0.001
Yes	15 (10)	3 (20)	12 (80)			
Minimal internal nutrition						
No	114 (76)	71 (62)	43 (38)	0.15	0.04 – 0.51	0.003
Yes	36 (24)	33 (92)	3 (8)			

179 # Row percentage, * Column percentage PROM-premature rupture of membrane, VAP-ventilator associated
180 pneumonia, ET-endotracheal tube
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3.3 Model performance

The goodness of fit for the model was tested by Receiver Operating Characteristics (ROC) curve plot. The area under the ROC curve was 0.929 for the model.

3.4 Antibiotic use pattern and antibiotic sensitivity pattern of pathogenic isolates

All neonates included in the study were prescribed antibiotics. The mean duration of antibiotics prescribed was 8.2 days. Most (78%) of the patients were prescribed a combination of two antibiotics, while 19% patents received a combination of three antibiotics. The most common antibiotic prescribed was amikacin (67%), followed by cefotaxime (30%), ceftriazone (29%), ampicillin (25%), meropenen (24%), vancomycin (20%), ampicilin with cloxacillin (14%), piperacillin with tazobactum (8%), ceftazidime (4%) and metronidazole (3%). The most common combination of antibiotics used was a third generation cephalosporin with amikacin (59%). Around 20% of the sick neonates were shifted to last resort antibiotics like meropenam and vancomycin after initially starting other antibiotics. The antibiotic sensitivity pattern of the isolates is shown in Table 2. In 58% of cases in our study gram-negative organisms caused blood stream HAI. *Klebsiella pneumonia* (24%), *Pseudomonas aeruginosa* (13%), *Escherichia coli* (11%), *Staphylococcus aureus* (21%), and Group B Streptococci (17%) were the prominent isolates. Around 73% of *Klebsiella pneumonia* and 80% of *Escherichia coli* were ESBL producing. The MDR rates were 86%, 82%, 80%, and 92% for *Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli* and *Proteus spp.* respectively. The MRSA proportion among the *S. aureus* isolates was 35%.

Table 2 Antibiotic-resistance-pattern of most prevalent causes of blood stream infections in a cohort of 150 neonates

Antimicrobial class/agent tested	**Activity by organism (number tested)**					
	K. pneumonia	***P. aeruginosa***	***E. coli***	***Proteus* sp**	***S. aureus***	***CONS***
	(11)	**(6)**	**(5)**	**(4)**	**(10)**	**(8)**
	R (%)	**R (%)**	**R (%)**	**R (%)**	**R (%)**	**R (%)**
Ampicillin	93	-	97	93	80	98
Amoxicillin/clavulanate	86	-	90	88	79	94
Piperacillin/tazobactam	40	74	44	44	-	-
Cefuroxime	82	-	93	86	-	-
Ceftriaxone	76	-	79	38	-	-
Cefixime	88	-	96	40	-	-
Ceftazidime	70	88	82	33	-	-
Ciprofloxacin	88	90	77	75	-	-
Norfloxacin	86	-	74	-	-	-
Ofloxacin	86	-	68	72	-	-
Levofloxacin	-	-	-	-	30	20
Gentamicin	66	69	69	42	-	-
Amikacin	39	38	32	19	20	18
Chloramphenicol	62	76	74	2	-	-
Tetracycline	94	94	91	71	-	-
Co-trimoxazole	90	84	94	87	-	-
Cefoxitin	-	-	-	-	35	-
Vancomycin	-	-	-	-	0	0
Imipenam	-	-	-	-	02	02

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4. Discussion

Surveillance per se has the potential to reduce HAI as it forces health care workers to at least think of behavioral change needed for reducing HAIs [1]. According to the World Bank India spends only 4.6% of its gross domestic product on health care [25]. Other health care problems take priority and LBCI surveillance is not considered a priority. But significant expenses in time and resources are needed for LBCI surveillance. Also, expertise is needed in study design, analysis and interpretation of results.

Over one-third of the global child mortality is accounted for by neonatal death [26]. Neonatal infections as a cause of death range from 25 to 71% in studies from India and Kenya [26,27]. In our study all newborns that died were suspected to have an LBCI. This suggests that HAI are a major contributor to neonatal mortality among admitted neonates.

Our study reported an incidence of neonatal LCBI sepsis as 6 per 1000 live hospital-born babies. This rate is less compared to the pooled rate of 21.4 per 1000 catheter days reported from four LMIC's in South America [28]. A review of neonatal infections among admitted patients reported infection rates between 6.8 to 38 per 1000 live births. The incidence of culture positivity in South Asia was 15 per 1000 live births [7].

Also, the rates reported in the present study are similar to the rates reported by the Delhi Neonatal Infection Study (DeNIS) collaboration study, which reported an incidence of total sepsis as14% and of culture-positive sepsis as 6·2% (95% CI 5·8–6·6) [9]. Our rate of LCBI in the preterm babies was 44%. BSI rates have been shown to vary from 21% to 39% in nurseries in LMICs, which admit mixed population of low and high-risk infants [29].

The high rate of neonatal infections in LMIC's strongly suggests lack of adequate hygiene in peri-partum and neonatal period in the hospitals [30]. With hospitals in the LMICs turning in hotbeds for HAIs the expected benefit of the safe institutional deliveries is getting severely undermined [30].

The incidence of neonatal sepsis is nearly nine times more in preterm babies with birth weight between 1000gm to 1500gm compared to term babies [4,7]. Pneumonia and meconium aspiration syndrome (MAS) are common causes of respiratory distress in neonates in resource constraint settings [31]. In our study the risk for BSI was higher for neonates with MAS, however there is no evidence that use of antibiotics for MAS reduces the rate of sepsis in neonates born through meconioum stained amniotic fluid [31]. A cohort study reported 3.5 times increased risk of sepsis in premature rupture of membranes in premature neonates [32], compared to our study which reported 7.5 times increased risk.

Previous studies have shown that very low birth weight and longer duration of antibiotic therapy were risk factors for umbilical arterial catheter-related sepsis [33]. A study from China showed that the incidence of umbilical venous catherization related septicemia was 9.5% [34]. The most common organisms associated with umbilical catheterization related septicemia in our study were *Staphylococcus aureus* and Coagulase-negative Staphylococcus, which is similar to that reported by previous studies [33,34]. Endotraceal intubation has its own complications because of its invasive nature. The risk of infection following endotreacheal intubation has not been studied very often in neonates except for VAP. However, there is evidence that endotreacheal intubation should be considered as an important risk for sepsis in neonates [4,7]. It is well known that mechanical ventilation is associated with increased risk of BSI, due to its invasive nature, breech in aspesis during and after the procedure, and during intratracheal suctioning [3,10,35]. The NICU enviorment per-se increases the risk of HAI and thus it is no surprise that duration of NICU stay was associated with increased risk of BSI's. The NICU's are considered as important focus areas for HAI surveillance and antibiotic stewardship [1,5,6,12]. Minimal enteral nutrition and early total enteral nutrition has been show to reduce risk of sepsis and duration of hospital stay [36].

There is paucity of data on bacterial causes of neonatal sepsis in neonates especially from resource-constrained settings [8]. *Klebsiella species, Escherichia coli, Staphylococcus aureus*, and Group B Streptococci (GBS) predominate in early-onset neonatal sepsis [8]. Whereas, late onset sepsis is predominantly caused by Gram-positive bacteria [8]. Gram-negative rods are responsible for 60 to

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70% of the blood culture positive infections in the neonatal period [8,35]. In our study *Klebsiella pneumoniae* was responsible for 22% of the LCBIs. This rate falls in the range of 16 to 28% reported from different parts of the world [37]. *S. aureus* is responsible for 8 to 22% of the bloodstream isolates in different regions [9]. Antibiotic resistance is common in the HAI isolates for NICU's. In Indian studies most common reported pathogens are gram-negative bacteria and most are resistant to ampicillin and gentamicin and some resistant to cephalosporins [9-11]. Antibiotic resistances to common first line agents make treatment costly and force the health care workers to go up in the spiral of antibiotic use. In our study we observed considerable variation in the type of antibiotics use in the NICU. It can be assumed that a considerable proportion could be irrational and overuse. However, if antibiotic use pattern is seen in light with the resistance pattern of bacterial isolates for HAI, the use is largely justified.

Methodological considerations

Our study has some weaknesses: 1) Hand hygiene is the most important intervention to reduce HAI in NICU. We did not measure adherence to hand hygiene as that was not an objective of the study. 2) Despite doing a literature review before the start of the study to identify risk factors for HAI, we could have missed out on some risk factors for the HAI in our study. Our study has following strengths: 1) Standard definition for the HAI surveillance were used for the surveillance which is needed for comparison of results with other international and national studies. 2) The study assessed the AST pattern for pathogens responsible for HAI, which in general is lacking in other published studies of HAI. 3) We have defined the level of care and have reported the LCBI rates according to the term and preterm, which is usually lacking in published studies from LMICs.

5. Conclusions

Since, very little is known about epidemiology of HAI in NICU's located in resource-limited settings including India, this study determined the prevalence, onset, risk factors and causative agents of HAI. The results can be used to identify high-risk neonates for HAI in Indian NICUs. The study highlights need to strengthen surveillance for HAI in resource-limited set-ups. More studies are needed to study the effect of interventions on the modifiable risk factors for HAI.

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