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Original Article

Neonatal septicemia: Its etiological agents and clinical associates

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Abstract

Aim: To identify the common bacterial pathogens associated with neonatal sepsis and to study their systemic, focal, and hematological associates. **Design:** Retrospective hospital-based observational study. **Setting:** Level II neonatal intensive care unit. **Subjects:** Totally, 87 neonates with culture proven sepsis. **Materials and Methods:** Case records of admitted neonates (from January 2013 to December 2013) with culture-positive sepsis were reviewed. Data were collected in a predesigned proforma and analyzed for type of bacterial isolate, and clinical and hematological manifestations of sepsis. **Results:** Blood culture was positive in 36.8% (87/236) of the neonates with sepsis. Among the culture-positive cases, 58.62% (51/87) were male, and 51.72% (45/87) were preterm babies. *Staphylococcus aureus* was the most commonly isolated organism (51, 58.62% cases), followed by *Klebsiella* in 14 (16.09%), coagulase-negative *Staphylococcus* in 6 (6.89%), *Acinetobacter* species in 4 (4.59%), *Escherichia coli* in 4 (4.59%), *Citrobacter freundii* in 2 (2.29%), *Pseudomonas aeruginosa* in 2 (2.29%), and *Candida* species in 2 (2.29%) babies. **Conclusions:** *S. aureus* was the predominant pathogenic organism in both inborn and outborn babies while *Klebsiella* was the main culprit for causing sepsis in outborn babies.

Key words: *Clinical, Microbial, Neonate, Sepsis*

Neonatal infections are estimated to cause about 1.6 million deaths worldwide and 40% of all neonatal deaths due to sepsis occur in developing countries [1]. Conventionally, neonatal sepsis has been classified as early onset sepsis (EOS) and late onset sepsis (LOS) with 72 h of life as a common demarcation. In contrast to bacteremia (bacteria in blood), septicemia usually consists of bacteremia plus a constellation of signs and symptoms caused by microorganisms or their toxic products in the circulation. There may be a progression of bacteremia to septicemia characterized by clinical manifestations. General signs of neonatal sepsis are lethargy/hypotonia, tachycardia/bradycardia, fever/hypothermia, abdominal distension, and hypotension/delayed capillary refill time, apnea, retractions, and grunting, and increased ventilator requirements. Focal infections associated with sepsis are pneumonia, meningitis, necrotizing enterocolitis (NEC), and urinary tract infections. Superficial infections such as conjunctivitis and oral thrush are not usually included under neonatal sepsis.

In developed countries, Group B *Streptococcus* and coagulase-negative staphylococci (CONS) are the most common etiological agents for EOS and LOS, respectively [2]. Definite diagnosis of sepsis is made by isolation of the organism in blood specimen culture. The bacteriological profile of neonatal sepsis is constantly changing with advances in the early diagnosis and treatment of neonatal sepsis. There are scanty regional data that studied an association between pathogenic organism and focal or systemic involvement [3,4]. Thus, we planned this study with the aim to determine various microbial agents of neonatal septicemia and their association with systemic, hematological, and focal infections in babies admitted to our neonatal intensive care unit (NICU).

MATERIALS AND METHODS

The study was conducted at the NICU of pediatric department of a teaching hospital of central India. This was a retrospective study conducted over 1-year from January 2013 to December 2013. Permission from Institutional Research and Ethical Committee was taken before commencement of the study. Only culture-positive septic neonates, both inborn and outborn, aged <1 months were included in the study. Babies who died within 3 days of admissions were excluded from the study. Clinical, demographic, and laboratory data were collected from the medical records of these neonates. As per our hospital protocol, septic screening including complete blood counts (CBC), C-reactive protein (CRP) estimation, and blood culture was done for all the symptomatic neonates. Following definitions were used to recruit babies in the study as mentioned in Table 1.

Clinical sepsis was defined as neonate having symptoms or signs of sepsis e.g. lethargy, fever/hypothermia, tachycardia/bradycardia, abdominal distension, and hypotension/delayed capillary refill time, apnea, or increased ventilatory requirement. Probable sepsis was clinical sepsis with a positive septic screen (CBC, CRP) and definite sepsis was defined as clinical sepsis with the growth of causative organism in blood culture. EOS was defined as clinical manifestations of sepsis appearing within 72 h of birth, while in LOS, clinical manifestations of sepsis are seen after 72 h of birth. CRP was considered positive when it was elevated to above 3 mg/dl. Anemia was taken as hemoglobin level <10 mg/dl, while leucopenia was total white blood cell count <4000/mm^3 and thrombocytopenia was defined as platelet count <100,000/mm^3. Hypoglycemia was taken as a random blood sugar of <40 mg/dl.

All the blood cultures were collected from a peripheral vein with proper aseptic precautions before starting any antibiotic therapy. The skin site was cleansed with 70% alcohol and povidone iodine (1%) followed by 70% alcohol again. 2 ml of blood was withdrawn and injected in the anaerobic broth bottles then incubated at 37°C for 7 days and observed daily for any turbidity due to bacterial growth. Subculture was done on sheep blood agar and MacConkey Agar routinely after 48 h and 7 days. Subculture was also done in between if visible turbidity appeared.

Information was collected regarding organism isolated, demographic profile, type of sepsis (EOS/LOS), presentation (non-specific/systemic), and hematology. Data were entered on predesigned proforma and then on Microsoft Excel sheet. Chi-square test was used and p<0.05 was considered significant.

RESULTS

During the study period, total 965 babies were delivered in our hospital and out of these, 206 babies required admission in NICU. During the same period, 79 outborn babies were also got admitted in our NICU with a total of 285 neonatal admissions. Inborn neonates were between 0 and 5 days old while outborn babies were between 1 and 30 days old. Out of 285 babies, clinical sepsis was present in 270 neonates (189 inborn and 71 outborn babies). Hence, 270 neonates underwent septic screen (CRP, hematological parameter) which was positive in 90% (259/270) of the cases, and these were labeled as probable sepsis. Out of 259 babies, blood culture reports were available for 236 neonates only while in remaining 22 neonates culture was either not sent or not available. Blood culture showed growth of organism only in 36.8% (87/236) of the neonates. Elevated CRP was seen in 74 (85%) out of 87 culture-positive babies.

As shown in Table 2, the male-to-female ratio was 1.4:1 among the culture-positive cases. Out of 87 neonates, 23 were outborn. 48 (55.1%) cases had early-onset sepsis, while 39 (44.8%) had late-onset sepsis. Less than half of the culture-positive neonates were delivered by normal delivery (48.2%, 42/87). Preterm babies were more prone for EOS (p<0.001) according to our observations. Of the 87 culture-positive cases, 7 (8%) died with slightly higher mortality in neonates with EOS than in LOS (4 vs. 3).

Table 1: Distribution of newborn admitted in neonatal intensive care unit

Newborn	Total admissions	Clinical sepsis (%)	Probable sepsis (positive CBC, CRP) (%)	Definite sepsis (blood culture-positive) (%)
Inborn	206	198 (96)	189 (91.7)	64 (31.0)
Outborn	79	72 (91.1)	70 (88.6)	23 (29.1)
Total	285	270 (94.7)	259 (90.8)	87 (30.5)

CBC: Complete blood counts, CRP: C-reactive protein

Table 2: Demographic details of culture-positive neonate included in study

Demographic	87 (100%)	EOS n=48 (55.1%)	LOS n=39 (44.8%)	p value
Male	51 (58.62)	32 (66.67)	19 (48.72)	0.091
Female	36 (41.37)	12 (25)	24 (61.54)	0.0005
Inborn	64 (73.5)	37 (77.0)	27 (69.2)	0.409
Outborn	23 (26.4)	9 (18.75)	14 (35.9)	0.0711
Full term	42 (48.27)	7 (14.59)	35 (89.75)	0
Preterm	45 (51.72)	32 (66.67)	13 (33.34)	0.002
AFD	77 (88.5)	40 (83.34)	37 (94.89)	0.092
SFD	10 (11.4)	7 (14.59)	3 (7.7)	0.317
NVD	42 (48.2)	31 (64.59)	11 (28.21)	0.0007
LSCS	49 (56.3)	12 (25)	37 (94.89)	0
Mortality in inborn	4/66 (6.0)	2 (4.17)	2 (5.13)	0.833
Mortality in outborn	3/23 (13.0)	2 (4.17)	1 (2.57)	0.6818

EOS: Early onset sepsis, LOS: Late onset sepsis, NVD: Normal vaginal delivery, LSCS: Lower segment caesarean section, SFD: Small for delivery, AFD: Average for delivery

Polymicrobial etiology was found in 5 babies out of 87 culture-positive babies hence total microbial isolates became 92. Detailed etiology of the 92 isolates is shown in Table 3. The most common isolates were *Staphylococcus aureus* in 58.7% (54/92) neonates followed by *Klebsiella* in 15.23% (14/92) cases. The case fatality rate was highest in neonates infected with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and least with *S. aureus* and *Escherichia coli* septicemia. Case fatality was not influenced by gender.

On Gram-staining, Gram-positive cocci were isolated from 67.3% (62/92) and Gram-negative bacilli in 32.7% (30/92) cases.

Staphylococci and *Klebsiella* were the most common Gram-positive and Gram-negative organisms, respectively. CONS were isolated in inborn babies mainly and recovered from only one outborn neonate. *S. aureus* was the predominant isolate in both inborn as well as outborn babies while *Klebsiella* has a significant association ($p<0.05$) with sepsis in outborn babies (Table 3).

Non-specific features of sepsis were seen in 47.1% (41/87) of culture-positive septic neonates, while rests were having one or more focal or systemic manifestations of sepsis as shown in Table 4. Most common presentation was pneumonia in 21 babies, where *S. aureus* was recovered in 10 babies, and *Klebsiella* was seen in 8 babies. Culture-positive NEC was seen in 9 babies and most of them had *Klebsiella* infection (44.4%). *S. aureus* was the main causative organism for meningitis, while the significant association was seen with *Acinetobacter* species ($p<0.05$) with meningitis. Table 5 shows that thrombocytopenia was the most common hematologic derangement seen in microbiologically confirmed sepsis.

DISCUSSION

In our study, male and female ratio was 1.4:1, and it could be attributed to gender bias in presentation to hospital for care. This is similar to the results of previous studies which reported a higher incidence of neonatal septicemia in males ranging from 59% to 82% [4]. Preterm babies were more prone for EOS ($p<0.001$) according to the results of our study. This is in contradiction to results shown by Hornik et al. who reported that preterms encounter LOS more than the EOS [5].

In the present study, 30.5% of the neonates with clinically suspected sepsis had positive blood culture which is in collaboration with the results of previous studies which reported positive blood culture in 26-50% of the cases [6-9]. Sepsis cannot always be excluded even when blood cultures turn out to be negative. Conversely, isolation of bacteria in a blood culture may reflect asymptomatic bacteremia or contamination. Furthermore, about 26% of all neonatal sepsis could be due to anaerobes where blood culture may be negative [10].

In our study, *S. aureus* was the predominant isolate (79.0%) in both inborn and outborn babies which is in agreement with few studies from India [11,12]. On the contrary, most of the Indian studies showed *Klebsiella* as the predominant pathogen in neonatal sepsis [13,14]. It has been proposed that EOS occurs by vertical transmission and is mainly caused by Gram-negative bacteria that are acquired before or after delivery, while LOS takes place by horizontal transmission and is principally associated with Gram-positive bacteria acquired after delivery, and source can be nosocomial or community-acquired infection [15].

Table 3: Bacterial isolates causing neonatal sepsis

Organisms	n=92 (%)	Inborn n=67 (%)	Outborn n=25 (%)	p value
Staphylococcus aureus	54 (58.7)	43 (64.1)	11 (44)	0.070
Klebsiella	14 (15.23)	7 (10.45)	7 (28)	0.037
CONS	6 (6.6)	5 (7.47)	1 (4)	0.556
Acinetobacter	4 (4.35)	3 (4.48)	1 (4)	0.928
Escherichia coli	4 (4.35)	2 (2.99)	2 (8)	0.288
Pseudomonas aeruginosa	4 (4.35)	3 (4.48)	1 (4)	0.928
Enterococcus	2 (2.2)	2 (2.99)	0	0.385
Citrobacter	2 (2.2)	1 (1.5)	1 (4)	0.458
Candida	2 (2.2)	1 (1.5)	1 (4)	0.458

CONS: Coagulase-negative staphylococci

Table 4: Various microbes and their association with clinical manifestations of sepsis

Organisms	Pneumonia (%)	NEC (%)	Shock (%)	DIC (%)	Meningitis (%)	NNHB (%)	p value
Total (92)	21 (24.13)	9 (10.34)	13 (14.94)	7 (8.04)	11 (12.64)	16 (18.3)	
Staphylococcus aureus	10 (47.6)	2 (22.2)	5 (38.46)	2 (28.57)	6 (54.54)	11 (68.75)	0.232
Klebsiella	8 (38.09)	4 (44.4)	3 (23.07)	2 (28.57)	0	2 (12.5)	0.114
CONS	1 (4.76)	1 (11.1)	0	0	1 (9.09)	1 (6.25)	0.832
Enterococcus	1 (4.76)	0	0	1 (14.28)	1 (9.09)	0	0.494
Citrobacter	0	1 (11.11)	1 (7.69)	1 (14.28)	0	0	0.335
Acinetobacter	0	0	3 (23.07)	0	2 (18.18)	0	0.034
Escherichia coli	2 (9.52)	0	1 (7.69)	1 (14.28)	0	0	0.542
Pseudomonas aeruginosa	0	1 (11.11)	0	0	1 (9.09)	2 (12.5)	0.421

NEC: Necrotising enterocolitis, DIC: Disseminated intravascular coagulation, NNHB: Neonatal hyperbilirubinemia, CONS: Coagulase-negative staphylococci

Table 5: Hematological parameters of cases in relation to microorganism growth

Total n=92	Anemia n=9 (%)	Leucopenia n=4 (4.59%)	Thrombocytopenia n=13 (14.94%)	Hypoglycemia n=8 (9.19%)	p value
Staphylococcus aureus	3 (33.3)	3 (75)	7 (53.8)	5 (62.5)	0.481
Klebsiella	3 (33.3)	1 (25)	4 (30.7)	3 (37.5)	0.975
CONS	0	0	0	0	0
Enterococcus	0	0	0	0	0
Citrobacter	0	0	0	0	0
Acinetobacter	1 (11.1)	0	2 (15.3)	0	0.59
Escherichia coli	0	2 (50)	0	0	0.001
Pseudomonas aeruginosa	1 (11.1)	0	0	0	0.413
Candida	0	0	1 (7.6)	0	0.645

CONS: Coagulase-negative staphylococci

Our study showed that in outborn babies, *Klebsiella* was the predominant causative organism ($p<0.05$). Case fatality rate was high in neonates having *Klebsiella* and *P. aeruginosa* sepsis [15]. *P. aeruginosa* was isolated mainly from inborn low birth weight babies requiring prolonged NICU stay. This is in agreement to results shown by Gras-Le Guen et al. who showed that extremely low birth weight neonates have significantly increased risk of acquiring *P. aeruginosa* in comparison to higher birth-weight infants [16].

Non-specific features of sepsis were seen in 47.1% of the culture-positive septic neonates, while the rest had one or more focal or systemic manifestations. In our study, pneumonia was the most common focal infection followed by meningitis, and NEC. Although, only in 2 of 11 babies with meningitis blood culture yielded *Acinetobacter*, but it has a significant association ($p<0.05$) with meningitis. This is in agreement to results shown by Fernandez-Viladrich et al. who showed that nosocomial meningitis is not an infrequent manifestation of *Acinetobacter* infection [17].

The result of this study demonstrated that thrombocytopenia was a more common presentation in culture-positive sepsis as compared to leucopenia but is not an organism-specific marker and this is consistent with previous reports of Manzoni et al. [18]. In our study, CONS were isolated from cultures of inborn babies mainly and only one neonate acquired it from the community. This is in agreement to results shown by Huebner and Goldmann that CONS infections are transmitted more among hospitalized patients. Most of these infections are hospital-acquired, and studies over the past several years suggest that they are often caused by strains that are transmitted among hospitalized patients with indwelling catheters or central lines [19].

This study was limited by small sample size, its retrospective nature, lack of information on presence and duration of central catheters, and sensitivity of microorganisms to various antibiotics. The spectrum of organisms that cause neonatal sepsis changes over time and varies from region to region and hospital to hospital even in the same city/country. Hence, we recommend further large multicentric prospective studies on clinical and bacteriological profile of neonatal sepsis.

CONCLUSION

S. aureus was the predominant pathogenic organism in both inborn and outborn babies, while *Klebsiella* was the main culprit of sepsis in outborn babies. Further, large multicentric prospective studies on clinical and bacteriological profile of neonatal sepsis should be undertaken as the spectrum of a causative organism changes over time and varies from region to region.

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