Original Article

Bacterial profile of Early versus Late onset neonatal sepsis and its antimicrobial susceptibility: A 1-year retrospective study in a tertiary level teaching hospital of Nepal

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Abstract

Introduction: Neonatal mortality accounts for 46% of all under-five deaths globally. The Neonatal Mortality Rate in Nepal (21/1,000 live births) still exceeds the global average. Infection remains one of the leading causes. South-Asian studies report different spectrum of pathogens in neonatal sepsis compared to Western literature, with concerns for emerging antimicrobial resistance. Local epidemiological data and periodic surveillance provides guidance to formulate antibiotic policy. This study was conducted to evaluate the organisms of neonatal sepsis and antimicrobial sensitivity pattern in a hospital setting.

Methods: A retrospective study was conducted in Neonatal Intensive Care Unit, Tribhuvan University Teaching Hospital from April, 2016 – April, 2017. All neonates treated as neonatal sepsis were screened. Only the cases with positive blood culture were enrolled.

Results: Out of 172 neonates with Probable sepsis, 36% cases were "Culture-proven sepsis". Majority were preterm (56.45%), Low Birth Weight (59.67%) and male (64.5%). Around 30% were outborn babies which probably contributed to high (75.8%) cases of Late-onset Neonatal Sepsis (LoNS). Besides Coagulase-Negative-Staphylococcus, antimicrobial-susceptible, Gram-negative organisms dominates Early-onset Neonatal Sepsis (EoNS). There were 53.19% Multidrug resistant (MDR) organisms in LoNS– Klebsiella followed by Pseudomonas, accounting for 64.7% deaths. Excluding Methicillin Resistant Staphylococcus aureus (MRSA), all LoNS associated MDR isolates were susceptible to Colistin. Majority (90%) Non-MDR isolates in LoNS were susceptible to Amikacin and Piperacillin-Tazobactum or Meropenem combination. All MRSA isolates were susceptible to Vancomycin or Teicoplanin.

Conclusion: Late onset neonatal sepsis remains the major burden. MDR isolates increase the challenge of newborn care with limited antimicrobial options and high mortality.

Keywords: Antimicrobial susceptibility, Bacterial profile, Neonatal sepsis

Introduction

The neonatal period is the most vulnerable phase of early childhood. Globally, 2.6 million neonatal deaths (46% of all under-five deaths) occurred during 2016.[1] Nepal Demographic and Health Survey (NDHS) 2016 data suggests that the neonatal mortality rate in Nepal has declined to 21 deaths per 1,000 live births in the most recent 5-year period.[2] Although below the regional (South-East Asia) average of 24.3, the rate still exceeds the global average of 19.2.[1] Given the existing healthcare disparities in newborn care among countries, the incidences and causes of neonatal mortality differ. Infection remains one of the leading causes in developing

nations which accounts for about 75% of the burden.[3] The causes of neonatal deaths in Nepal are infection, birth asphyxia, preterm birth, congenital anomalies and others.[4]

Neonatal sepsis is broadly classified into "Early onset (<72 hours)" and "Late onset (>72 hours)" based on the onset of presentation after birth.[5] This classification guides clinical management since the micro-organisms and the pathogenesis involved differ. Emerging literature highlights different spectrum of pathogens in India and South-Asian countries compared to Western literature.[6] In addition, the changing pattern of organisms and the threat of antimicrobial resistance has been a growing concern in developing countries.[7]

Consensus from international experts in 2008, proposed standardized international terminologies to define resistant organisms - Multidrug-resistant (MDR), Extensively drug-resistant (XDR) and Pandrug-resistant (PDR). MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and PDR is defined as non-susceptibility to all agents in all antimicrobial categories. In cases of incomplete testing, bacterial isolates can only be characterized as "possible MDR", "possible XDR" or "possible PDR".[8]

In view of variable characteristics of micro-organisms, local epidemiological data and periodic surveillance provides guidance to formulate antibiotic policy. Therefore, this study was conducted to evaluate the causative organisms of neonatal sepsis and their respective antibiotic sensitivity pattern in a hospital environment. Considering the growing concerns for increased resistance, our departmental policy avoids Cephalosporin as an empirical treatment of neonatal sepsis.

Methods

After approval from Institutional Review Board, this retrospective study was conducted in Neonatal Intensive Care Unit (NICU), Neonatology Division, Department of Pediatrics, Tribhuvan University Teaching Hospital (TUTH) from 1st Baisakh 2073 till 31st Chaitra, 2073 (13th April, 2016 – 13th April, 2017). It is a 578- bedded central, tertiary-care, multispecialty teaching hospital located in Kathmandu, Nepal. The annual deliveries are around 4,500-5,000; the majority being high-risk pregnancies. The hospital is a major center for referral of high-risk pregnancies and sick newborns from all over the nation. Additionally, a Birthing Center has been operational in recent years. The hospital is also a site for Undergraduate medical, Postgraduate Pediatrics and MDGP (MD General Practitioner) training under Tribhuvan University, with Level III (6 beds) and Level II (8 beds) NICU services, besides MD, DM and MCh courses in various specialities.

All neonates, irrespective of inborn or outborn status, managed in NICU as neonatal sepsis during a one-year period were screened. A total of 172 neonates had "Probable sepsis", out of which, 62 cases were "Culture proven sepsis". Case definition for neonatal sepsis (Probable sepsis and Culture-proven sepsis) was according to the standard definition.[9] Only the cases with positive blood culture were enrolled and the relevant data collected.

As per microbiological standards for culture, 1 ml of blood was drawn using aseptic precautions before starting antibiotic treatment and inoculated directly into Brain Heart Infusion Broth (BHI) manufactured by HiMedia Pvt. Ltd., India, in a ratio of blood: BHI of 1:5. Then the processing and incubation of the sample was done. The antimicrobial susceptibility test (AST) was done by Kirby-Bauer disk diffusion technique in compliance with Clinical Laboratory Standards Institute (CLSI) recommendations.[10]

Results

During the study period, there were 4,828 deliveries and 618 admissions (including 91 Outborn babies) in NICU (Level II and III, combined). A total of 172 (27.8%) admitted newborns were treated for "Probable sepsis", out of which, 62 (36%) babies had positive blood cultures. Majority of the culture-proven cases were Preterm (35, 56.45%) compared to Term (27,

44.54%). Low Birth Weight (LBW) neonates were 37 (59.67%) and that with normal birth weight were 25 (40.32%). Male (40, 64.5%) outnumbered female babies (22, 35.4%). Forty-three (69.3%) babies were Inborn whereas 19 (30.6%) were Outborn. Of all cases with positive blood cultures, EoNS and LoNS constituted (15, 24.1%) and (47, 75.8%), respectively. The organisms isolated in EoNS were (Coagulase negative Staphylococcus) CONS (7), Pseudomonas aeruginosa (2), Staphylococcus aureus (2), E. coli (2), Citrobacterfreundii (1) and Acinetobacterbaumanii (1). Twelve (80%) of these isolates were susceptible to antimicrobials, whereas 1 isolate of Pseudomonas aeruginosa and 2 E. coli isolates were Possible MDR as shown in Table 1. Majority (13, 86.66%) of newborns with EoNS did not have identifiable risk factors. Likewise, the isolates of LoNS were Klebsiella pneumoniae (19), Pseudomonas aeruginosa (6), Acinetobacter baumanii (5), CONS (5), Burkholderia cepacia (5), E. coli (3), Staphylococcus aureus (2) and Enterococcus spp. (2). Twenty-five (53.19%) of these isolates were MDR, 5 (10.63%) were Possible MDR and the rest 17 (36.17%) were susceptible isolates as depicted in Table 2. Of the MDROs associated with LoNS, 15 (60%) were Klebsiella, 3 (12%) Pseudomonas aeruginosa and E. coli each, 2 (8%) Staphylococcus aureus, 1 (4%) Enterococcus and Acinetobacterbaumanii each. Overall survival in culture positive cases was 67.74% (42). Among mortalities, EoNS and LoNS accounted for 15% (3) and 85% (17) deaths, respectively. MDR isolates resulted in 55% (11) overall mortalities whereas Possible MDR isolates resulted in 15% (3) and Susceptible isolates in 30% (6) mortalities. Eleven (64.7%) of LoNS related deaths were associated with MDR isolates, 3 (17.6%) each with Possible MDR and Susceptible isolates, respectively. All deaths in neonates with EoNS were associated with susceptible organisms. Almost 87% of the isolates in EoNS were susceptible to empirical first line antibiotics (Ampicillin and Amikacin), and the combination of Ciprofloxacin and Amikacin increased the coverage to 100%. (Table 3)

Table 1 Antimicrobial resistance pattern of bacterial isolates in Early onset sepsis

Organisms	Susceptible	Possible MDR	MDR	Total
Pseudomonas	1	1	0	2
E. coli	0	2	0	2
Citrobacter	1	0	0	1
Acinetobacter	1	0	0	1
Staph. aureus	2	0	0	2
CONS	7	0	0	7
Total	**12**	**3**	**0**	**15**

Table 2 Antimicrobial resistance pattern of bacterial isolates in Late onset sepsis

Organisms	Susceptible	Possible MDR	MDR	Total
Klebsiella	2	2	15	19
Pseudomonas	1	2	3	6
Acinetobacter	3	1	1	5
Burkholderia	5	0	0	5
CONS	5	0	0	5
E. coli	0	0	3	3
Staph. aureus	0	0	2	2
Enterococcus	1	0	1	2
Total	**17**	**5**	**25**	**47**

Excluding Methicillin Resistant Staphylococcus aureus (MRSA), all MDR isolates associated in LoNS were susceptible to Colistin. (Table 4) Ninty % of Non-MDR, including Possible MDR isolates in LoNS were susceptible to Amikacin and Piperacillin-Tazobactum or Meropenem combination. All MRSA isolates were susceptible to Vancomycin and Teicoplanin.

Table 3 Antibiogram of blood culture isolates of Early onset neonatal sepsis

S – Sensitive, **NT** – Not Tested, **NR** – Non Relevant

Number of Resistant isolates not shown in the table. It can be calculated from {n-(S+NT)}

Antibiotics	Pseudomonas (n=2)		E.coli (n=2)		Citrobacter (n=1)		Acinetobacter (n=1)		Staph. aureus (n=2)		CONS (n=7)	
	S	NT	S	NT	S	NT	S	NT	S	NT	S	NT
Ampicillin	0	1	0	0	0	0	0	0	NR	NR	3	0
Cefotaxime	0	2	0	2	0	1	0	1	NR	NR	NR	NR
Cefepime	0	2	0	2	0	1	0	1	NR	NR	NR	NR
Ceftazidime	0	2	0	2	0	1	0	1	NR	NR	NR	NR
Ciprofloxacin	1	0	1	1	1	0	1	0	1	1	4	1
Levofloxacin	1	1	1	1	1	0	0	1	NR	NR	NR	NR
Gentamicin	1	1	0	2	0	1	0	1	0	2	2	5
Amikacin	1	0	1	1	0	0	1	0	2	0	5	2
Piperacillin	0	2	0	2	0	1	0	0	0	2	NR	NR
Piperacillin-Tazobactum	0	1	1	1	0	1	0	1	0	2	NR	NR
Meropenem	1	1	0	2	1	0	0	1	0	2	NR	NR
Imipenem	0	2	0	2	0	1	0	1	0	2	NR	NR
Colistinsulphate	1	1	0	2	0	1	0	1	NR	NR	NR	NR
Polymyxin B	1	1	0	2	0	1	0	1	NR	NR	NR	NR
Tigecycline	0	2	0	2	0	1	0	1	0	2	NR	NR
Cloxacillin	NR	NR	0	2	0	1	NR	NR	2	0	4	2
Vancomycin	NR	NR	NR	NR	NR	NR	NR	NR	0	2	6	1
Teicoplanin	NR	NR	NR	NR	NR	NR	NR	NR	0	2	2	5
Chloramphenicol	NR	NR	0	2	0	1	0	1	0	2	NR	NR
Cotrimoxazole	NR	NR	1	1	1	0	1	0	0	2	0	6

Table 4 Antibiogram of blood culture isolates of Late-onset neonatal sepsis

R – Resistant, **NT** – Not Tested, **NR** – Not Relevant

Number of susceptible isolates not shown. It can be calculated from {n-(R+NT)}

Antibiotics	Klebsiella (n=19)		Pseudomonas (n=6)		Acinetobacter (n=5)		Burkholderia (n=5)		CONS (n=5)		E. coli (n=3)		Staph. Aureus (n=2)		Enterococcus (n=2)	
	R	NT	R	NT	R	NT	R	NT	R	NT	R	NT	R	NT	R	NT
Ampicillin	11	8	3	3	5	0	2	3	5	0	2	1	NR	NR	1	0
Cefotaxime	1	18	2	4	1	4	0	5	0	4	2	1	NR	NR	NR	NR
Cefepime	4	15	0	5	1	4	0	5	0	5	0	3	NR	NR	NR	NR
Ceftazidime	5	14	1	4	2	3	0	3	0	5	0	3	NR	NR	NR	NR
Ciprofloxacin	6	9	3	1	1	4	5	0	1	2	2	1	0	0	1	0
Levofloxacin	9	7	2	3	1	4	3	2	0	4	0	3	0	2	0	2
Gentamicin	1	16	0	3	0	4	2	3	1	3	1	2	0	2	1	0
Amikacin	11	8	2	0	1	0	4	1	0	3	3	0	0	0	1	1
Piperacillin	0	19	0	6	0	5	0	5	NR	NR	1	2	0	2	NR	NR
Piperacillin-Tazobactum	7	11	2	3	0	3	2	3	NR	NR	2	1	0	2	NR	NR
Meropenem	1	14	0	4	1	2	1	1	NR	NR	3	0	0	2	0	1
Imipenem	12	5	1	5	1	3	0	5	NR	NR	0	3	0	2	0	2
Colistinsulphate	0	0	0	0	0	0	NR	NR	NR	NR	0	0	NR	NR	NR	NR
Polymyxin B	0	0	0	0	0	0	NR	NR	NR	NR	0	0	NR	NR	NR	NR
Tigecycline	2	7	0	4	0	3	0	5	NR	NR	0	1	NR	NR	0	1
Cloxacillin	NR	NR	NR	NR	NR	NR	NR	NR	0	3	NR	NR	2	0	0	2
Vancomycin	NR	NR	NR	NR	NR	NR	NR	NR	1	2	NR	NR	0	0	0	0
Teicoplanin	NR	NR	NR	NR	NR	NR	NR	NR	1	4	NR	NR	0	0	0	1
Chloramphenicol	1	9	0	6	0	5	0	5	NR	NR	0	2	0	2	0	2
Cotrimoxazole	2	16	1	5	0	5	0	0	NR	NR	0	3	0	2	0	2

Discussion

In our study, neonatal sepsis is one of the leading causes of significant mortality across all gestation, birth weight and gender. LoNS accounts for the majority of neonatal deaths. It is an alarming situation to note that the isolates of LoNS are predominantly MDR, with limited antimicrobial alternatives.

Blood culture is considered as the gold standard for diagnosing neonatal septicemia. Conventional method based blood culture positivity in this study was only 36%. It, however, is relatively a larger yield compared to other studies (using the same culture methods) in Nepal which ranges from 12.6% - 30.8%.[12, 13] The technical limitations of the manual culture systems and prior antibiotic exposure in outborn cases may have an effect on the yield in our study. Culture yield seems to improve with automated systems. Hasan AS et al, in his study of 101 matched pairs of blood culture from neonates in NICU, showed that BacT/Alert 3D system had significantly improved yield compared to manual method (45.5% vs 18.8%) along with rapid median time to positivity (11.5 h vs 24 h).[14] Surase PV et al also reported significantly higher recovery of positive culture on comparing BACTEC 9050 against conventional blood culture (32% vs 19.88%).[15]

Consistent with known vulnerability to sepsis, the incidence of neonatal sepsis in this study was higher in preterm and low birth weight babies compared to their counterparts. The similar findings were demonstrated in studies done by Shrestha RK et al[13] and Shah GS et al.[15] Also, the predilection to sepsis was noted in male neonates. Such result could either reflect the inherent risk for sepsis among males as suggested by Dutta S et al[16] in his study or the preferential health attention for males by families in our society.[17]

Being a major national referral center for high risk deliveries, our neonate population in NICU is predominantly preterm who are known to be at risk of acquiring infection. Access for NICU admission available to outborn referrals including babies in severe sepsis, also increase the total burden of sepsis and the risk of cross-infection which explains our findings of high proportion of LoNS. Majority of the isolates associated with LoNS were Gram negative organisms. However, several studies conducted in academic-oriented hospitals of Nepal with in-hospital birthing facility and open to outborn cases, contradict our findings; Staphylococcus aureus was the most common organism.[13, 18, 19] Neither was Staphylococcus aureus, in our study, a common isolate as Gram negative organisms were, as noted by Shrestha S et al in their study done in Dhulikhel hospital.[20] Indian National Neonatal Perinatal database[21] and a recent Indian population-based prospective study done in a 12,622 births in Odisha state[22] also differs in this regard that Staphylococcus aureus is a common organism for LoNS.

EoNS accounted for 24% of culture positive cases in our study. Gram positive organisms were the dominant pathogens, among which CoNS was the commonest. Although Anshari S. et al[12] had similar finding, several other studies from Nepal[20] and earlier Indian National Neonatal Perinatal database[21] reported Gram negatives as the leading isolates in blood cultures of EoNS. Available evidences from recent Indian studies, however, vary: Staphylococcus aureus are the predominant organisms for EoS in hospital setting[6], whereas Gram negative organisms predominate in community setting.[22] Western literature emphasizes Group B Streptococcus (GBS) as the leading cause of EOS despite widespread prophylaxis for GBS prevention.[23] On the contrary, our study found none. Such contradicting result incurred in institution where the recommendation of late antenatal GBS screening for all pregnant women is not strictly practiced. The suboptimal results from existing culture methods causing discrepancies cannot be disregarded since a study[24] noted significant difference in yield of GBS on comparing different culture methods against CDC recommended selective enrichment broths.

CoNS is a common skin commensal, primarily acquired from the environment after birth in neonates. Therefore, CoNS isolate in a single blood culture can be a mere contaminant especially if the sterility of sampling technique is poor. Some studies suggest two simultaneous blood cultures to help rule out contamination.[25,26,27] Struthers S. et al observed that using a single culture alone, rather than two, would have resulted in a 31% increase in the number of babies diagnosed with CONS infection. He also further added that the positive predictive value and the specificity of first culture alone for a diagnosis of CoNS was 0.76 and 0.94 respectively, when compared to the results from a combination of two blood cultures.[25] The risk of CoNS infection increases with invasive interventions and parenteral nutrition.[28] In our study (single set blood culture policy for evaluation of sepsis), although, CoNS was the predominant organism isolated, none had undergone invasive interventions. Healy CM. et al

observed that true CoNS infection is unlikely in infants with birth weight > 2000 g and gestation > 34 weeks.[29] In the present study, only 1 out of 7 cases categorized as EoNS with CoNS isolates, was below 34 weeks of gestation. Considering all the factors stated above, the possibility of majority of CoNS isolates being contaminants cannot be denied. So, excluding CoNS isolates, Gram negative organisms predominate EoNS in our study as well. Hence, a cautious interpretation of cultures seems imperative.

Eighty percentage of isolates in blood culture of EoNS were non-MDR organisms. The remaining isolates were categorized as "Possible MDR" in view of incomplete antimicrobial susceptibility testing. Eighty-seven percentage of the bacterial isolates in EoNS were susceptible to the first line antibiotics (Ampicillin and Amikacin), and the combination of Ciprofloxacin and Amikacin increased the coverage to 100%. Although most studies lack an elaboration of antimicrobial susceptibility patterns based on the onset of neonatal sepsis, Ansari S. et al observed that a substantial proportion of isolates in EoNS were resistant to the first line combination of antibiotics. Around 33.3 % were MRSA.[12] Contrary to the traditional recommendations on the use of Ciprofloxacin in neonates, studies appear to support its use.[30, 31, 32]

Around 54% of bacterial isolates in LoNS were MDR, 10% were Possible MDR and the rest 36% were susceptible isolates. Excluding Methicillin Resistant Staphylococcus aureus (MRSA), all MDR isolates associated with LoNS were susceptible to Colistin. Based on the available antimicrobial sensitivity results in the present study, 90% of Non-MDR, including Possible MDR isolates were susceptible to Amikacin and Piperacillin-Tazobactum or Meropenem combination. All MRSA isolates were susceptible to Vancomycin and Teicoplanin. Comparison with other studies was limited because of unavailability of sepsis categorization based susceptibility testing. The few identified studies with categorization has limited spectrum of sensitivity testing on the other hand.

Overall survival in culture positive cases was 67.74% (42). Among mortalities, EoNS and LoNS accounted for 15% (3) and 85% (17) deaths, respectively. MDR isolates resulted in 55% (11) overall mortalities whereas Possible MDR isolates resulted in 15% (3) and Susceptible isolates in 30% (6) mortalities. Eleven (64.7%) of LoNS related deaths were associated with MDR isolates, 17.6% (3) with Possible MDR and Susceptible isolates each.

Conclusion

Late onset neonatal sepsis remains the major burden. MDR isolates increase the challenge of newborn care with limited antimicrobial options and high mortality. The bacteriological profile varies across different locations and even within the same region which highlights the need for local epidemiological data. The retrospective nature of the study excluding neonates in general wards (Non-NICU set up) and incomplete antimicrobial testing are the limitations. Large scale prospective studies in our population may help define the true situation of neonatal sepsis and guide the antibiotic policy.

Conflict of interest: None declared.

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