Extended Spectrum Beta Lactamase producing Gram negative bacteria - An emerging menace in Neonatal Sepsis.

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Abstract

Introduction: Neonatal sepsis caused by extended spectrum beta lactamase (ESBL) producing Gram negative bacteria (GNB) is associated with significantly high mortality and morbidity. Clinical features and risk factors for such neonatal sepsis can help in identifying it early.

Objectives: Aim of the study was to estimate the incidence, risk factors, clinical features and antibiotic sensitivity of GNB and outcomes of ESBL GNB in neonatal sepsis.

Methodology: A prospective observational conducted at regional tertiary care health center. Statistical analysis was carried out with SPSS version 23.0. Results: A total of 87 cases of Gram negative neonatal sepsis were included in study. Male: female was 1.7:1. Forty nine (56.3%) isolates were ESBL positive strains. The clinical features in order of frequency were shock, lethargy, sclerema, disseminated intravascular coagulation and severe thrombocytopenia. Out born neonates (p=0.03), late onset sepsis (p=0.05) and mechanical ventilation (p=0.002) were the risk factors for ESBL GNB sepsis. Mortality associated with ESBL sepsis was 26.5%. Carbapenems and Piperacillin + Tazobactum were most sensitive antibiotics and high resistant for cephalosporins was observed.

Conclusion: ESBL GNB neonatal sepsis is an emerging threat with high mortality in Neonatal Intensive care unit.

Key words: Antibiotic sensitivity, extended spectrum beta lactamase, Gram negative neonatal sepsis, Risk factors of ESBL.

Introduction:

Neonatal sepsis is potentially fatal blood stream infection occurring in the first month of life[1].The incidence of neonatal septicemia caused by Gram negative bacilli (GNB) is on increasing trend in the past decade[2]. It is associated with higher morbidity, mortality, longer duration of neonatal intensive care unit (NICU) and hospital stay with additional financial burden on family, and poor long terms outcomes[2,3]. The emergence of multi-drug resistant among gram negative bacilli specifically Extended spectrum β-lactamase (ESBL) producing GNB is concerning due to limited treatment options available for multiple drug resistant (MDR) strain. An inappropriate initial antibiotic is the predisposing factor for neonatal severe sepsis and its poor outcomes[4]. Sepsis related mortality is largely preventable with timely recognition of sepsis and earlier use of rational antimicrobial therapy[5]. ESBL infection is primarily nosocomial infection with outbreaks, but recently community acquired ESBL infections are on rise due to improper usage of antibiotics. Acenetobacter is the most commonly isolated and colonizing GNB from ICUs. Patient to patient transmission seen primarily in NICU is due by the healthcare workers[6]. Presently, there are not many studies from India concerning ESBL GNB neonatal sepsis, hence this study was conducted to estimate the incidence, risk factors, analyse antibiotic sensitivity patternss and outcomes of ESBL septicemia in NICU.

Materials And Methods:

A prospective observational conducted at regional tertiary care health center. Sample size was calculated using Open Epic Version 2.3.1. Using previous study estimates, the proportion of neonates with ESBL producers was 67.3%[7] i.e p=67.3% at 10%

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absolute precision with 95% confidence level sample size was calculated to 87. The study period was one year. All the neonates admitted to NICU with at least one blood culture positive for GNB at any time during hospital stay were included in the study. Detailed demographic profile, All information including place of birth, admission symptomatology, perinatal risk factors of sepsis and, clinical signs and outcome with diagnosis were recorded in a predesigned proforma. The details of clinical features and various risk factors such as birth weight, gestational age, outborn/inborn, premature rupture of membrane, preeclampsia/eclampsia, oligohydroamnios, birth asphyxia, meconium/foul smelling liquor, mechanical ventilation, early onset sepsis (EOS)/late onset sepsis (LOS) were recorded. Days on mechanical ventilation and continuous positive pressure ventilation (CPAP) were recorded. Onset of sepsis was divided into early onset sepsis and late onset sepsis as per World Health Organisation/National Neonatal Forum.

To identify risk factors for ESBL producing GNB, all enrolled patients were dichotomized into: **Septicemic patients due to ESBL GNB and Septicemic patients due to non ESBL GNB**. Complete haemogram with CRP was sent with blood cultures and reports were noted. Thrombocytopenia was divided into mild (1-1.5 lakh/cumm), moderate (0.5-1 lakh/cumm) and severe (<50000/cumm). Lecocytosis was defined as corrected total white blood cell (WBC) count >20,000/cumm and leucopenia was defined as total WBC count <3500/cumm. CRP was considered positive if it was >6.0mg/dl. ESBL production was screened and confirmed in all isolates with double disc diffusion test according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2015[8].Antibiotic sensitivity pattern for gram negative bacilli, screening and confirmation for ESBL production in GNB was done as per standard protocol.

Statistical analysis:

Categorical variable were compared by using CHI2 test or Fisher's exact test. T-test had been used to compare significant difference between two means. Statistical analysis was done with SPSS software version 23.

Results:

During the study period 1180 neonates were admitted. We isolated in 87 (7.3%) neonates, gram negative bacilli (GNB), among these 49 (56.3%) ESBL producing gram negative bacteria were isolated from blood culture, showing ESBL sepsis in 49 neonates.

In our study,

- Term babies were more (56/87) compared to Preterm babies (31/87).
- Male to female ratio was1.7:1.
- Total out-born were 41 (47.1%) and inborn were 46 (52.8%).
- Out of 87 cultures positive, Gram-negative neonatal sepsis are 38(43.7%) were 2.5-2.9 Kg neonates. Only 19 (16.53%) neonates were below 1.5 Kg birth weight in the study.
- Out of 87 cases of Gram-negative neonatal sepsis 13 mothers (14.9%) were having Prolonged rupture of membranes (>18 hours). Oligohydramnios was seen in 6 mothers (6.9%). Preeclampsia/eclampsia was seen in 13 mothers (14.9%). No mother had been found having fever or chorioamnionitis as risk factors for neonatal sepsis.
- Birth asphyxia was present in 25 neonates (28.7%) whereas meconium-stained liquor and foul-smelling liquor was present in 28 (32.2%) and 7 (8%) cases respectively.

Table 1: Baseline characters and risk factors ESBL / Non ESBL GNB (N=87)

	ESBL (n=49)	Non ESBL (n=38)	P value
Birth weight <1.5KG	13	6	0.29
Gestation age <34 weeks	17	8	0.08
Inborn/out-born	21/28	25/13	0.03
Male/Female	31/18	24/14	0.9
EOS/LOS	22/27	25/13	0.05
Perinatal risk factors			
PROM	2	5	0.7
Preeclampsia or eclampsia	4	9	0.3
Oligohydroamnios	3	3	0.7
Birth Asphyxia	18 (36.71%)	7 (18.4%)	0.06
Meconium stained liquor	18	10	0.3
Foul smelling liquor	4	5	0.1
Mechanical ventilation	30 (61.2%)	10 (26.3%)	0.002

- Early onset sepsis (n=47, 54%) was more in our study. Out of 87 cases, 49 cases were ESBL sepsis which showed 13 deaths (ESBL sepsis mortality - 26.5%), 25 improved and discharged and 11 went against medical advice.

- We found that death was significantly higher with ESBL GNB sepsis and the study shows that Out-born neonates, Late onset sepsis and Mechanical ventilation were significant risk factors for ESBL GNB sepsis.

Table 2: Clinical features - ESBL and Non ESBL GNB (N=87)

Signs	ESBL (n=49)	Non ESBL (n=38)	P
Tachycardia	43 (87.8%)	23 (60.5%)	0.003
Tachypnea	9 (18.4%)	13 (34.2%)	0.09
Poor perfusion	40 (81.6%)	12 (31.6%)	<0.001
Poor CTA	15(30.6%)	3 (7.9%)	<0.001
Sclerema	16 (32.7%)	4 (10.5%)	0.01
DIC	8 (16.3%)	1 (2.6%)	0.03
Laboratory findings			
Leucocytopenia	12 (24.5%)	6 (15.8%)	0.4
Leukocytosis	6 (12.2%)	3 (7.9%)	0.4
Neutropenia	8 (16.3%)	2 (5.3%)	0.09
Thrombocytopenia	34 (69%)	11 (28.9%)	<0.001
DEATH	13 (26.5%)	1 (2.6%)	0.009

- In our study tachycardia, poor perfusion, poor cry-tone-activity, sclerema, disseminated intravascular coagulation and thrombocytopenia were clinical features associated with ESBL GNB neonatal sepsis
- We also observed that severe thrombocytopenia (platelet counts <50,000/cumm) was seen in 19 (21.8%) cases. We compared severe thrombocytopenia and ESBL GNB sepsis and found significant association (p=0.005). Days on mechanical ventilation was significantly higher for ESBL GNB sepsis (p=0.01) and significantly higher CRP values observed for ESBL GNB sepsis (p<0.001)
- Antibiotic sensitivity testing showed high resistant to 3rd generation cephalosporins. Sensitivity to Carbapenems (Imipenem and Meropenem) was almost 100% for all GNB except one Klebsiella species isolate which was resistant to both imipenem and meropenem, probable a carbepenamase producer. Resistant was observed for fluoroquinolones (levofloxacin and Ofloxacin) almost similar to cephalosporins. Piperacillin + Tazobactum combination showed average 70% sensitivity, specifically against ESBL producing GNB.

Discussion:

In this study we found that ESBL producing gram negative septicaemia was higher in NICU and it is associated with higher morbidity and significantly higher mortality. We found that 68% (17/25) of neonates below 34 weeks of gestation and 68.4% (13/19) of neonates below 1.5Kg birth weight were associated with ESBL GNB infection. Pessoa-Silva CL et al and Abdel-Hady H et al observed low birth weight and preterm neonates are risk factors for ESBL GNB infections [9,10]. However, in our study we found no statistical significance. Non-significant results can be explained by higher number of term neonates and birth asphyxia cases in our study. It can also be explained by proper nursing care and newer equipment which may protect smaller infants from ESBL GNB infections.

In our study none of the maternal risk factors i.e., premature rupture of membrane (PROM), preeclampsia/ eclampsia, chorioamnionitis, maternal fever, meconium-stained liquor and foul-smelling liquor were associated with ESBL GNB sepsis in neonates. These are consistent with findings of studies conducted by Roy S et al and Tsai et al[11,12]. Gaurav J et al and Sehgal R et al showed that PROM is significantly associated with ESBL GNB infections in neonates which is opposite to the results we observed[13,14].

We observed that higher association of birth asphyxia with ESBL GNB sepsis in our study though statistically not significant. Similar results were observed by Tsai et al, who showed higher incidence of ESBL septicemia in neonates with low APGAR score at 5 min (<7)[15]. This can be explained by increased interventions and need for ventilation support with birth asphyxia neonates which can increase the risk of ESBL GNB infection.

In our study we found that 68.3% out-born neonates and 67.5% late onset sepsis had ESBL GNB sepsis. Results were similar to Tsai MS et al and Vijayakanthi N et al[12,16]. Higher rate of out-born neonates with ESBL infections and higher association of LOS and ESBL can be suggestive of high prevalence of ESBL producing bacteria in community.

We observed significantly higher ESBL GNB infection in neonates on mechanical ventilation (p =0.001). Abdel-Hady H et al and Tsai et al observed similar results[10,12].

The clinical features of ESBL sepsis in order of frequency were tachycardia, poor perfusion, poor cry-tone activity, sclerema and disseminated intravascular coagulation. Gaurav J et al showed association of ESBL GBN sepsis with lethargy and shock[13]. Tsai et al showed that shock within 48 hours of onset of sepsis is clinical feature of ESBL GNB sepsis[14].

In our study thrombocytopenia was significantly associated with ESBL GNB sepsis. Even more severe thrombocytopenia (<50,000/cumm) was

seen significantly high in ESBL infections compared to non ESBL inactions. Arif SH et al observed that thrombocytopenia is commonly seen with Gram positive bacteria but severity of the thrombocytopenia is more in gram negative septicaemia[17]. Srinivasan R et al showed association between ESBL Klebsiella and thrombocytopenia. Their study showed that severe thrombocytopenia in all neonates infected with ESBL Klebsiella and required platelet transfusion[18].

Higher mortality rate was observed in our study as compared to other study. Higher mortality can be explained by failure to recognize ESBL GNB infections and delayed change in antibiotics.

Antibiotic sensitivity testing showed meropenem and imipenem were most sensitive antibiotics specifically for ESBL producing GNB. 3rd generation cephalosporins were resistant for almost 1/3 of cases. Levofloxacin and Ofloxacin were sensitive to almost half of the isolates. This shows cross resistant between cephalosporins and fluoroquinolones. Piperacillin and Tazobactam were sensitive to almost 2/3 isolates. ESBL GNB isolates were most sensitive to Carbapenems followed by Piperacillin and Tazobactam combination. Gray JW et al showed that Piperacillin + Tazobactum combination provide better Gram negative coverage than penicillin + aminoglycoside combination[19]. Piperacillin + Tazobactam has been used successfully to treat preterm infant with blood stream infection of ESBL producing K. pneumoniae (Pillay t et al)[20].

Conclusion:

Extended spectrum beta lactamases producing gram negative bacilli are associated with severe neonatal sepsis. Shock, lethargy, sclerema and disseminated intravascular coagulation are clinical features associated with ESBL GNB neonatal sepsis. Severe thrombocytopenia (<50,000/cumm) and higher mortality rates are seen significantly associated with ESBL GNB neonatal sepsis. Paediatricians should keep in mind about emerging resistant to 3rd generation cephalosporins which also associated with cross resistant with fluoroquinolones

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