Neonatal sepsis is a disseminated disease with positive blood culture during the first month of life and is a common cause of mortality in neonates. The microbial etiology of neonatal sepsis is variable and an increase in sepsis caused by gram negative organisms has been reported in the recent years. The emergence of cephalosporin resistance in gram-negative nosocomial pathogens is a formidable problem, associated with adverse clinical outcomes and increased hospital costs. Methodology: The study was conducted in the Department of Microbiology, JNMCH, AMU, Aligarh between January 2006 to September 2011. Blood culture was done on 1,740 samples received from neonatal intensive care unit (NICU), Department of Paediatrics. Cultures showing growth were identified using standard biochemical procedures and antimicrobial sensitivity was done by Kirby bauer disc diffusion testing. Detection of ESBL production was done as per the CLSI recommendations. Results: A total of 997 (57.3%) samples showed growth, out of which 455 (28.2%) samples showed growth of gram positive pathogens and in 542 (31.1%) Gram-negative bacteria were isolated. Male to female ratio was 0.6:1, with majority patients having early onset sepsis. *Klebsiella pneumoniae* was the predominant isolate 350 (66.05%). 43.1% of the gram negative isolates showed resistance against cephalosporins and 37.5% were ESBL producers. Conclusions: Gram negative bacteria are an important cause of early and late-onset neonatal sepsis. Screening for ESBL should be done routinely in all the hospitals to prevent dissemination of these strains within and between hospitals.

**Key words:** Bacteraemia, Antimicrobial resistance, Cephalosporins.

Neonatal sepsis is a disseminated disease with positive blood culture during the first month of life and is a common cause of mortality in neonates. Mortality rates in neonatal sepsis differ according to the type of organisms involved. Gram negative bacteria cause the highest mortality rate in neonatal sepsis. The microbial etiology of neonatal sepsis is variable and often changes temporarily. *Group B Streptococci* is a common cause of neonatal sepsis in west but infrequent in India and other tropical countries. *Staphylococcus aureus, Klebsiella, E.coli* along with *Coagulase negative Staphylococcus* species and *Pseudomonas* are the main organisms responsible for neonatal septecemia in India. An increase in sepsis caused by gram negative organisms has been reported in the recent years. The variety and the antimicrobial sensitivity of the microorganism causing sepsis can be different for each NICU and it can also change over time for the same unit. Indiscriminate use of broad spectrum

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antibiotics and prolonged courses of antibiotic therapy have resulted in increased incidence of multi-drug resistant bacteria. Recently the emergence of cephalosporin resistance in gram-negative nosocomial pathogens is a formidable problem, associated with adverse clinical outcomes and increased hospital costs. This study was undertaken to evaluate the prevalence of gram-negative bacteraemia in neonates and to determine the level of cephalosporin resistance amongst these isolates.

**Methodology**

The study was conducted in the Enteric laboratory of the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh between January 2006 to September 2011. A total number of 1,740 samples were received from neonatal intensive care unit (NICU), Department of Paediatrics, for blood culture in brain heart infusion broth. Repeated subcultures were done on 5% sheep blood agar and MacConkey agar after 24 hours, 48 hours and 7 days of incubation at 37°C. Cultures showing growth were identified by standard biochemical procedures. Antimicrobial susceptibility testing was done on Mueller Hinton’s agar by Kirby Bauer Disc diffusion method as per the CLSI guidelines for the following antimicrobials: cefotaxime 30µg, ceftriaxone 30µg, cefoperazone 75µg, cefoperazone-sulbactum 75/75 µg, gentamicin 10µg, amikacin 30µg, tobramycin 10µg, ciprofloxacin 5µg, Imipenem 10µg.

**Screening for ESBL**

This was done as part of routine susceptibility testing, according to the following criteria. Two discs, ceftriaxone (30 µg) and cefotaxime (30 µg), were used. An inhibition zone of ≥ 25mm for ceftriaxone and ≥ 27mm for cefotaxime indicated that the strain probably produced ESBL.

**Phenotypic confirmation test for ESBL**

Two discs, containing cefoperazone (75 µg) and cefoperazone-sulbactum (75/75 µg), were used. A ≥ 5mm increase in zone diameter for cefoperazone along with sulbactum versus its zone when tested alone confirmed ESBL production.

**RESULTS**

Blood culture was positive in a total of 997(57.3%) patients. Four hundred and fifty five (26.2%) samples showed growth of gram positive pathogens and 542(31.1%) non-duplicate Gram-
negative bacteria were isolated from these blood samples. The male-to-female ratio was 0.6:1. Among patients with sepsis, 372 patients (68.63%) had early-onset and 170 (31.37%) had late onset neonatal sepsis.

Among the gram negative pathogens *Klebsiella pneumoniae* was the predominant isolate 358(66.05%) followed by *Escherichia coli* 84(15.5%), *Acinetobacter spp* 42(7.7%) and *Pseudomonas aeruginosa* 32(5.9%) and *Citrobacter* 26(4.8%).

Two hundred and thirty four isolates (43.1%) were found to show resistance against cephalosporins. Maximum resistance was observed in case of *Klebsiella pneumoniae* 161(68.80%), followed by *Acinetobacter* 23(9.82%), *E. coli* 22 (9.40%) and *Pseudomonas aeruginosa* 17(7.26%). Least resistance was observed in *Citrobacter*.11(4.70%).

**DISCUSSION**

Gram-negative bacteraemia is a major cause of sepsis in neonates, contributing substantially to the mortality and morbidity. This retrospective study on septicaemic neonates admitted to the NICU of J.N. Medical College, Aligarh, presents valuable data on sepsis caused by Gram-negative bacteria, over a five year period. Because sepsis may manifest with nonspecific clinical signs and its effects may be devastating, rapid empiric antibiotic therapy based on local pattern of aetiology and antimicrobial susceptibility is recommended.

In our study prevalence of gram negative septicaemia was found to be 31.1%, which is comparable to other studies from North India. In this study male to female ratio was 0.6:1 and early onset sepsis was more common than late-onset sepsis (68.63% versus 31.37%). This finding was comparable to the results of Kumar et al. The high prevalence of early onset sepsis in semitropical countries like India may be because of multi-resistant hospital acquired pathogens, which are transmitted during the perinatal period. These organisms are usually resistant genera of the family *Enterobacteriaceae*, *Pseudomonas spp*. and *Staphylococci*.

The most frequent isolates were *Klebsiella spp* (66.05%), *Escherichia coli* (15.5%), and *Citrobacter* (4.8%) among the *Enterobacteriaceae* and among the nil fermenters *Acinetobacter spp* (7.7%) and *Pseudomonas aeruginosa* (5.9%). Similar microbiological spectrum has been reported in other Indian studies. Cephalosporin resistance was noted in 43.1% of the isolates, out of which 37.5% isolates were ESBL producers. The high prevalence of ESBL-producing isolates may be due to the selective pressure imposed by extensive use of antimicrobials in the intensive-care unit. A study from central India reported even higher rate of ESBL production (76.5%).

Antibiotic resistance can cause many difficulties in the treatment of sepsis such as increase in mortality rate, duration of hospitalization and treatment expenses.

According to the result of this study, we can conclude that gram negative bacteria are an important cause of early and late-onset neonatal sepsis. ESBL producing strains are of major concern since treatment options of these strains are limited to drugs like imipenem, which are beyond the reach of the low socio-economic population. Efforts should be taken for justified and restricted use of antimicrobials to decrease the spread of antimicrobial resistance. Screening for ESBL should be done routinely in all the hospitals to prevent dissemination of these strains within and between hospitals.

**REFERENCES**

5. Shah SS, Ehrenkraut RA, Gallagher PG. Increasing incidence of gram negative rod bacteraemia in a


