Original Article

Neonatal Gram-Negative Bacteremia

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Abstract. A 22 months prospective study of neonatal gram-negative bacteremia was undertaken in a 15 bed NICU to find out the incidence and antibiotic resistance patterns. Clinically suspected 1326 cases of neonatal sepsis were studied during this period. More than 25% of the cases were microbiologically positive for sepsis. Among 230 (67.2%) cases of gram-negative bacteremia, the predominant isolates were *Pseudomonas aeruginosa* (38.3%), *Klebsiella pneumoniae* (30.4%), *Escherichia coli* (15.6%) and *Acinetobacter* sp. (7.8%). Fifty-nine per cent of the neonates were born in hospital while 41% were from community and referral cases.

Lower respiratory tract infection, umbilical sepsis, central intravenous line infection and infection following invasive procedures were the most commonly identified sources of septicemia. Prematurity and low birth weight were the main underlying conditions in 60% of the neonates. Total mortality was 32%. Increased mortality was mainly associated with neutropenia, nosocomial infection and inappropriate antibiotic therapy. Resistance was increasingly noted against many antibiotics. The isolates were predominantly resistant to extended spectrum cephalosporins (25%-75%), piperacillin (68%-78%), and gentamicin (23%-69%).

The commonest microorganisms causing gram-negative bacteremia were *Pseudomonas aeruginosa* followed by *Klebsiella pneumoniae*. The community-acquired bacteremia was mainly due to *E. coli*. The proportion of preterm and low birth weight babies was significantly high, and the major contributing factor in total mortality. Sensitivity to different antibiotics conclusively proved that a combination of ampicillin + sulbactam with amikacin or ampicillin + sulbactam with ciprofloxacin is most effective. **(Indian J Pediatr 2000; 67 : 27-32)**

Key words : *Antibiotic resistance; Bacteremia; Clinical isolates; Neonatal sepsis; Nosocomial infection.*

Gram-negative bacteremia is a major cause of sepsis in neonates, contributing substantially to the mortality and morbidity[1,2]. Septicemia due to *Pseudomonas, Klebsiella, Citrobacter* is commonly reported in neonates from various countries[3]. Gram-positive organisms are mainly responsible for early onset of infection whereas gram-negative organisms are mainly encountered during late onset of infection in neonates[3,4]. An incidence of nosocomial infections in neonates is also very high.

The sources for gram-negative septicaemia are mainly urinary tract infection (UTI), gastro-intestinal tract infection (GITI), and lower respiratory tract infection (LRTI). Neonatal sepsis is commonly associated with central intravenous catheters and the extensive use of antibiotics leading to high incidences of nosocomial infection[5].

Early identification of an organism and appropriate antibiotic treatment is essential to prevent the increasing mortality and morbidity. Many underlying conditions and diseases, however, should be considered alongwith possible sources of infection, invasive procedures including central intra-venous catheter, empiric therapy and host defence system while calculating morbidity and mortality. Personal hygiene of the staff in neonatal intensive care unit (NICU), and newborn babies and skin and umbilical stump care are very important in prevention of neonatal infection, particularly wound infection and septicemia[5,6,7].

The objective of this study was to analyse the incidence of gram-negative bacteremia and assess the antibiotic resistance patterns of the isolates in NICU.

MATERIALS AND METHODS

A prospective study of neonatal gram-negative bacteremia was undertaken between October 1994 through August 1996, at a 15-bed NICU, in Pune. An average annual admission of neonates in this centre is 1300. During the period, 1326 suspected cases of neonatal sepsis were studied.

The neonates were selected as septicemic based on the following clinical criteria : hyper-thermic or hypo-thermic, respiratory distress or spells of apnoea, bradycardia,

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abdominal distension, diarrhoea, refusal to feeds, vomiting, jaundice, lethargy, feeble cry, hyperflexia, hepatomegaly, skin rash, umbilical sepsis and other focal infections.

The data collected by daily surveillance of patients' charts included age, sex, underlying condition, predisposing factors, source of infection, therapy, complications, outcome, serum proteins and leucocytic count.

Amidst aseptic precautions, the venous blood was obtained and inoculated into two bottles (one for aerobic and second for anaerobic tryptic soya broth) which were incubated at 37°C. The presence or absence of visible growth was noted every 24 hours. The incubation period was 5 days to rule out late growing bacteria. Clinical isolates were identified by colonial morphology, microscopy, and routine biochemical reaction. The culture media were obtained from Hi-Media Ltd, Mumbai (India).

Antibiotic Susceptibility Testing (Antibiogram)

Antibiotic susceptibility was detected by disc diffusion method, as recommended by National Committee for Clinical Laboratory Standards[8]. Diagnostic sensitivity test (DST) agar plates were inoculated with a bacterial suspension in saline of standard density (0.5 McFarland), which had been prepared from a 24 h culture on blood agar. The antibiotic discs used were ampicillin/sulbactam (10 ug), amikacin (30 ug), cefoperazone (75 ug), ceftazidime (30 ug), ceftriaxone (30 ug), cefuroxime (30 ug), cefotaxime (30 ug), ciprofloxacin (5 ug), gentamicin (10 ug), piperacillin (100 ug), and tobramycin (10 ug).

The plates were examined after incubation of 18 h and 24 h at 37°C. The zones of inhibition were read and interpreted as per manufacturer's instructions.

Definitions

Neonatal sepsis : A clinical syndrome characterized by signs and symptoms of infection within first 28 days of life.

Source of infection : Focal infection associated with or without bacteremia often with a positive culture from that site.

Septic shock : High grade sepsis associated with hypotension, perfusion abnormalities and organ dysfunction.

Renal failure : Progressive oliguria with a rise in serum creatinine value in a patient with a previously normal renal function.

Respiratory failure : Respiratory distress resulting in acute hypoxemia or hypercapnia requiring mechanical ventilation.

Polymicrobial bacteremia : Growth from a culture material of more than one microbial pathogen, thought to be of clinical significance.

Neutropenia : Absolute neutrophilic count of <1000/cmm.

Prematurity : Gestational age <32 weeks.

Low birth weight : Weight <1500 grams at the time of birth.

Inappropriate antibiotic treatment : When antibiotic to which the isolated organism was susceptible in-vitro, was not administered empirically within 24 hours of sample collection.

Cardio-respiratory arrest : Clinical cessation of the heart and lungs function which leads to death.

Nosocomial infection : Infection acquired in an institutional (usually hospital or nursing home) setting. It normally includes unusual number of isolates of an uncommon pathogen.

Mortality : Death during hospitalization due to neonatal septicaemia.

RESULTS

Of the 1326 clinically suspected cases, 342 (25.8%) were culture positive of which 230 (67.2%) were gram-negative pathogens. Polymicrobial bacteremia (involving gram-negative organisms) was diagnosed in 4.8% (11 out of 230 cases).

Isolation of Organisms (Table 1)

The predominant isolates were *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli,* and *Acinetobacter* sp. The isolates of *Enterobacter, Proteus, Salmonella, Serratia,* and *Providentia* sp. were also observed but in lesser proportions.

Table 1. Isolates from Neonatal Gram-negative Bacteremia

Name of the organism	No. of isolates	Percentage
Pseudomonas aeruginosa	88	38.3
Klebsiella pneumoniae	70	30.4
Escherichia coli	36	15.65
Acinetobacter spp.	18	7.8
Enterobacter spp.	7	3.0
Proteus spp.	6	2.6
Salmonella spp.	2	0.9
Serratia spp.	2	0.9
Providentia spp.	1	0.45
Total	230	100

Table 2. Antibiotic Resistance Pattern of Predominant Isolates (n = 230)

Sr No.	Antibiotic	Abbreviation	Resistance pattern (%)			
			Klebsiella	*E. coli*	*Pseudomonas*	*Acinetobacter*
1.	Ampicillin/Sulbactum	As	32.8	33.8	30.0	23.6
2.	Piperacillin	Pc	57.2	62.5	67.8	66.7
3.	Cefoperazone	Cs	53.6	37.5	77.5	44.5
4.	Ceftazidime	Ca	60.8	56.3	67.8	66.7
5.	Ceftriaxone	Ci	75.0	50.0	77.5	66.7
6.	Cefuroxime	Cu	24.3	43.8	54.9	55.6
7.	Cephotaxime	Ce	35.8	25.0	77.5	44.5
8.	Amikacin	Ak	14.3	6.3	45.2	22.3
9.	Gentamicin	G	60.8	68.8	61.3	44.5
10.	Tobramycin	Tb	82.2	68.8	74.2	55.6
11.	Ciprofloxacin	Cf	24.0	19.4	32.8	32.8
12.	Resistant to all	All	10.7	6.2	22.2	22.2

Antibiotic Susceptibility Testing

The antibiotic resistance profiles of the four predominant isolates during two years were reviewed (Table 2). Most of the organisms were not susceptible to second and third generation cephalosporins. The percentage of antibiotic resistance was observed more in hospital-acquired than community-acquired gram-negative bacteria. A resistogram of main isolate from hospital-acquired and community-acquired bacteremia is shown in Fig. 1.

Pseudomonas aeruginosa was the main organism isolated from 88 (38.3%) blood samples. It was also isolated from 1 urine, 6 bronchial aspirates and 2 umbilical stump pus samples simultaneously with blood samples having similar antibiograms. A remarkable resistance was observed against extended cephalosporins (55-78%) and piperacillin (67.8%); moderate resistance to amikacin (45.2%) and ciprofloxacin (32.8%). A total of 22.2% of the isolates were resistant to all tested antibiotics.

Klebsiella pneumoniae was the second major organism isolated from 70 (30.4%) bacteremia cases. The isolates were susceptible to amikacin (85.7%), cefuroxime (75.7%), ampicillin + sulbactam (67.2%) and relatively less sensitive to other cephalosporins and aminoglycosides. Of the total isolates, 10.7% were resistant to all tested antibiotics.

E. coli was isolated significantly from the community-acquired bacteremia, although hospital-acquired isolates were relatively more resistant. The isolates were relatively susceptible to amikacin (93.7%), cefotaxime (75%), and less susceptible to all other extended cephalosporins and aminoglycosides tested. Total isolates which were resistant against the tested antibiotics accounted to 6.2%.

Acinetobacter was isolated from 18 (7.8%) clinical samples. This was mainly nosocomial and isolated from blood, although it was also observed from rectal swab (1), umbilical stump pus (2), and ascitic fluid (1) in addition to central I.V. catheters (2) and bronchial intubation (1). The organism was found susceptible only to amikacin (> 77%) and ampicillin + sulbactam (> 76%). Total 22.2% of the isolates were resistant to all tested antibiotics.

Age and Sex Incidence

The study was restricted to neonates. A high incidence of gram-negative bacteremia was noted between the age of 6 days to 17 days in neonates born in the hospital, as against 12 days to 25 days old neonates in community-borne cases. A slight male predominance was found in gram-negative bacteremia (55.5%).

Underlying Conditions and Predisposition

Factors like prematurity in 121 (52.6%) and low birth weight in 116 (50.4%) were major contributors towards increased mortality (Table 3). The neutropenia in 50 (22.6%), and hypoproteinaemia in 39 (16.9%) were the main precipitating factors associated with neonatal gram-negative bacteremia (NGNB). In these patients, the most common pathogens were *P. aeruginosa* and *K. pneumoniae*. Central intra-venous

catheters were responsible for 24 (10.5%) cases of bacteremia wherein the same organism was isolated from catheter and peripheral blood. The bacteremia following invasive procedures was observed in 29 (12.6%) cases, which included bronchial intubation, ventriculo-peritoneal shunt and trauma. *Acinetobacter* was an important isolate from these sites, in addition to *Pseudomonas* and *Klebsiella.*

Source of Infection

In the majority of cases no obvious clinical source was detected despite thorough investigations. In most cases the source of bacteremia was central intravenous catheters and infection following invasive procedures (Table 4). Local infection was found in 33 (14.4%) neonates. Other factors included LRTI, umbilical sepsis, abdominal, gastrointestinal and urinary tract infections. *Klebsiella* was the common pathogen in LRTI.

Hospital vs Community Acquired Bacteremia

Hospital-acquired gram-negative bacteremia accounted for 59% (136 of 230) against 41% (94 of 230) community-acquired cases and the former was significantly associated with mortality. The predominant organisms were *P. aeruginosa, K. pneumoniae* and *Acinetobacter* sp. *E. coli* was mainly isolated from community-acquired infection. Septic shock, respiratory distress syndrome, peritonitis were the main complications noted during the period.

Mortality was 32.2% (74 of 230 cases), relatively high as compared to developed countries[5,9,17]. The factors significantly associated were, prematurity, neutropenia, and nosocomial contamination. Death due to cardio-respiratory arrest with septicaemia was notably high (42 of 74). Other factors were acute respiratory distress syndrome, infection due to central intra-venous catheters, septic shock, polymicrobial sepsis. Thirty one (13.5%) neonates were on inappropriate antibiotic therapy at the time when cultures were taken. This also contributed to the mortality of 16.2% (12 of 74). The hospital-acquired bacteremia carried a significant risk of mortality than community-acquired (22.2% vs 10%).

DISCUSSION

Neonatal gram-negative bacteremia was detected in 67.2% of cases. The high proportion of nosocomial infection was significant in neonatal unit of our hospital, in contrast to hospitals elsewhere[6,9,12].

P. aeruginosa was the predominant organism. *K. pneumoniae* and *E. coli* were the second and third most common isolates respectively. Bacteremia due to *Klebsiella*[6], *E. coli, Enterobacter* and *Pseudomonas* has been reported in pediatric patients[9]. Anderson *et al* showed relative importance of *Enterobacter* bacteremia in pediatric patients[10]. Thus organisms causing neonatal septicaemia differ from place to place. Predominance of gram-negative septicemia has been reportedly as high as 92.5%[11], in contrast to gram-positive bacteremia[12,13]. Incidence of *Acinetobacter* infection in neonates has been reported by Horrevorts *et al*[14] and is a major problem in neonatal intensive care unit[15].

Table 2 presents antibiotic resistance profile against predominant isolates. *P. aeruginosa, E.coli, K. pneumoniae* and *Acinetobacter* sp. were responsible for high degree of resistance. All predominant isolates were sensitive to amikacin while relatively resistant to gentamicin and tobramycin. Most of the isolates were resistant to extended spectrum cephalosporins. These results confirm the earlier studies[16,17]. Newer cephalosporins, gentamicin, piperacillin and ampicillin + sulbactam were in extensive use when the study was undertaken. Thus the authors could not

Table 3. Main Underlying Conditions & Predisposition to Neonatal Gram-negative Bacteremia (n= 230)

Sr. No.	Underlying condition/ finding	Number of cases	Percentage
1.	Prematurity	121	52.6
2.	Low birth weight	116	50.4
3.	Neutropenia	50	22.6
4.	Hypoproteinaemia	39	16.9
5.	Bronchial intubation	18	7.8
6.	Ventriculo-peritoneal shunt	4	1.7
7.	Trauma	7	3.0

Table 4. Focal Infection and Sources of Gram-negative Bacteremia in Neonates

Source	No. of cases	Percentage
Lower respiratory tract	17	7.4
Umbilical sepsis/wound	9	3.9
Abdominal and gastrointestinal	6	2.6
Urinary tract	1	0.4
Central intravenous catheter	24	10.5
Post-invasive procedure	29	12.6
Unknown	144	62.6
Total	230	100

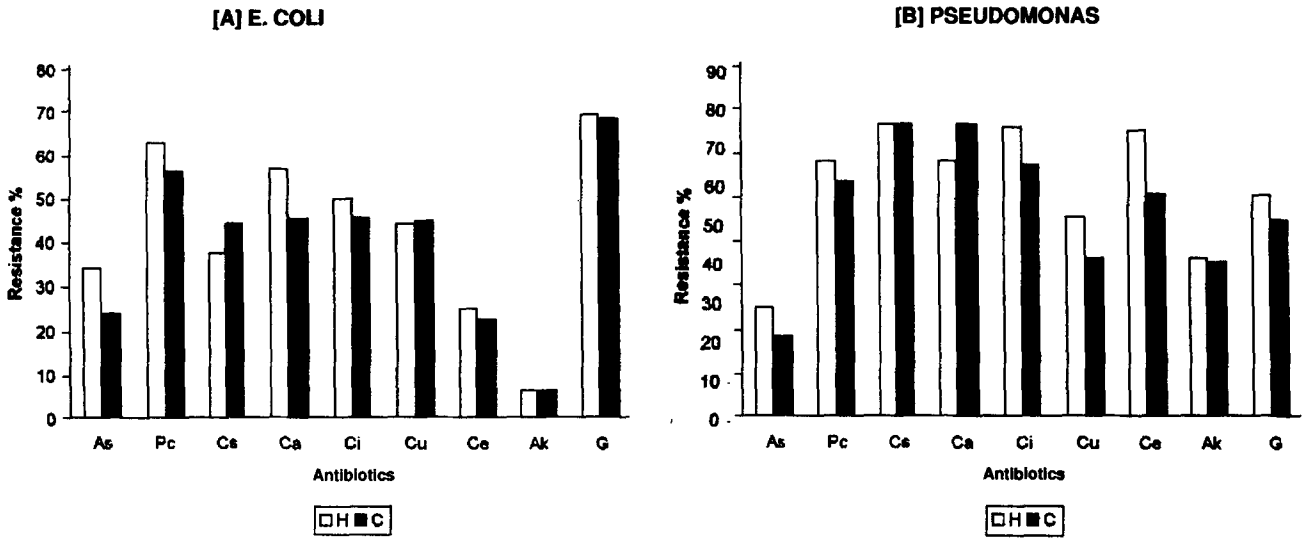

Fig. 1. Resistogram of main isolate from hospital-acquired (H), and community-acquired (C) neonatal gram-negative bacteremia.

demonstrate a significant change in antibiotic susceptibility pattern over the period of study. Resistance against modified penicillins and aminoglycosides in neonatal gram-negative bacteremia has been reported on earlier occasions also[11,17,18]. Fig. 1 shows the high levels of resistance among predominant isolates from hospital and community-acquired bacteremia.

Predisposition, Source of Infection and Mortality

The host factor plays an important role in prognosis of infection in neonates. This hospital is a main referral hospital for rural people and many small hospitals in the vicinity; low socio-economic class of patients are significantly high. Due to improper antenatal care and nutritional deficiencies prematurity and low birth weight are common factors. To some extent illiteracy and lack of personal hygiene, contribute to the nosocomial infection. Futhermore, a free use of empirical antibiotic therapy was common which has caused increasing resistance among nosocomials[6,9,16]. Polymicrobial infection was found associated with high mortality.

CONCLUSION

Neonatal gram-negative bacteremia was found in 67.2% (230 of 342) of neonatal sepsis cases and contributed to increasing mortality. No anaerobic organism was found associated with neonatal gram- negative bacteremia. Due to inappropriate initial antibiotic therapy, local sepsis was significantly associated with gram-negative septicaemia. There was a predominance of *Pseudomonas aeruginosa* (38.3%), followed by *Klebsiella pneumoniae* (30.4%), and *E.coli* (15.7%) in this hospital. Because of a relatively high rate of resistance to extended cephalosporins and penicillins, a combination of amikacin and ampicillin + sulbactam as empirical antibiotic therapy for gram-negative septicaemia could work well in these settings. Furthermore, the mothers should be taught the importance of antenatal care, nutrition and personal hygiene, so as to minimize the incidence of low birth weight babies and neonatal sepsis.

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PREVENTION OF ROTAVIRUS DISEASE : USE OF ROTAVIRUS VACCINE

Virtually all children experience rotavirus (Rv) infection before school entry. Data collected by the Centers for Disease Control and Prevention from 1979 through 1992 indicate that approximately 50000 hospitalizations attributable to Rv occur annually in the US, a number that approximates about 1 in 78 children being hospitalized with Rv diarrhoea by 5 years of age.

RotaShield (Wyeth-Lederle Vaccines and Pediatrics, Philadelphia, PA) was licensed by the FDA on August 1998 for oral administration to infants at 2, 4 and 6 months of age. The rationale for using Rv immunisation for prevention or modification of Rv is based on several considerations. First, the rate of illness attributable to Rv among children is comparable in industrialized and developing countries, which indicates that improved public sanitation is unlikely to decrease the incidence of disease. Second, although implementation of oral rehydration programs to prevent dehydration has improved in the US, widespread use is inadequate to prevent significant morbidity. Third, trials of rhesus rotavirus-tetravalent vaccine in the US, Finland, and Venezuela show ϵ ficacy rates of approximately 80% for prevention of severe illness and 48%-68% against Rv-induced diarrheal episodes. These results are similar to the protection observed after natural Rv infection, which also confers better protection against subsequent episodes of severe disease than mild illness.

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