Original article

Neonatal septicemia in NICU of a tertiary care center in north India due to extended spectrum beta lacta-mase (ESBL) producing bacteria

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Abstract

The study was designed to determine Extended-spectrum β-lactamase (ESBL) mediated resistance in Gram negative bacteria isolated from cases of neonatal septicemia. In this prospective study; blood culture from 260 neonates admitted with suspected sepsis to Neonatal Intensive Care Unit (NICU) of LLR Hospital, Kanpur, India were included. Clinical presentation of the cases and other details were recorded and two ml blood was collected from each patient in 8-10 ml brain heart infusion broth. The blood culture was done. All Gram negative organisms isolated were subjected to biochemical identification and antibiotic susceptibility testing. Screening for ESBL was done in all Gram negative isolates. Growth of one or more organisms was detected in blood samples of 142/260 cases (54.6% culture positive). (Table2). The commonest organism isolated was Candida spp. (22.5%). Followed by Gram-positive bacteria (34.5%) & Gram-negative bacteria (41.5 %). Antibiotic resistance pattern of ESBL Vs Non- ESBL producers was studied and clinical outcome was noted in each case. Majority of the Klebsiella (52.1%) and Enterobacter(50.0%) isolates were ESBL producing. Resistance to amikacin and ciprofloxacin was less in either group in both bacteria. Mortality was significantly higher in ESBL producers (34.8%) than non-ESBL producers (9.7%). In view of high prevalence and mortality assossiated with of ESBL producers indiscriminate use of third-generation cephalosporins and must be strongly discouraged as empirical drug and they should be used after sensitivity testing.

Keywords: Neonatal septecemia, gram negative bacteria, Extended-spectrum β-lactamase (ESBL)

Introduction

In India, septicemia is a leading cause of neonatal mortality next to perinatal asphyxia and occurs in a significant proportion of admissions in a Neonatal Intensive Care Unit (NICU).1 treatment protocol for sepsis management must be based on current knowledge of the causative organisms and their antibiotic sensitivity pattern.2 The gold standard for the diagnosis of sepsis is the isolation of the causative organism from a blood sample. The process takes 48 hours or more duration.3 Resistance to antimicrobials is more common in situations like NICU, where they are used very frequently and unjudiciously.4 The excessive use of third-generation cephalosporins as first-line drugs in these cases adds to our concern. Until the mid-1980s, resistance to β-lactam antibiotics was known to be limited to organisms with chromosomal β-lactamase genes which was not transmissible. The situation however got complicated further by isolation of multiresistant bacteria. Such multiresistance in Gram-negative bacteria may be due to production of extendedspectrum β-lactamase (ESBL). These different types of enzymes, called ESBLs, are produced exclusively by Gram-negative bacteria and are active against extendedspectrum cephalosporins, aztreonam, narrowspectrum cephalosporins and anti Gram negative- bacterium penicillins and the only treatment option left is carbapenems.5,6 Neonates are particularly vulnerable to infection, so any delay in the initiation of empirical ther-

apy or wrong choice of antibiotics may become hazardous. Keeping these facts in mind, the present study was carried out to know the prevalence and resistance pattern of ESBL-producing Gram negative bacteria in neonates.

Material and methods

A total of 260 neonates admitted with suspected sepsis to GSVM Medical College, Kanpur were prospectively enrolled for this study. Clinical presentation of the cases and other details were recorded. Two ml blood was collected from each patient aseptically. The samples were inoculated in 5 ml of brain heart infusion broth. Overnight incubation was done then broths were sub cultured on blood agar and MacConkey. Negative results were followed daily by examining the broth daily and a final subculture was done at the end of seventh day. Bacteria showing growth were identified by Gram staining, colony characters, and biochemical tests. Antimicrobial sensitivity was performed by disc diffusion method.The antibiotic discs used were Ampicillin (10 mg), amoxycillin/clavulanic acid (20/10 mg), gentamicin (10 mg), trimethoprim/sulfomethoxazole (1.25/23.75mg) Penicillin (10 units), cephalexin (30 mg), amikacin (30 mg), Erythromycin (5 mg), Vancomycin (30 mg), tobramycin (10 mg), ceftazidime (30 mg), ceftriaxone (30 mg), cefoperazone/sulbactam (75/30 mg). netilmicin (30 mg), tetracycline (30 mg), ciprofloxacin (5 mg)and chloramphenicol (30mg). Detection of ESBL was done in all the 119 Gram negative isolates by the criteria recommended by the NCCLS (National Committee for clinical laboratory Standards, 2005). Two discs, ceftazidime (30Dg) and ceftazidime /clavulanic acid (30/10Dg) were used. A zone enhancement of 5 mm indicated that the strain produced ESBL. Antibiotic resistance pattern of ESBL and non-ESBL producers was studied and clinical outcome was noted in each case.

Results

Of the total 260 cases of suspected neonatal sepsis, the most common clinical feature observed was refusal to feed and abnormality of the temperature (Table 1). Growth of one or more organisms was detected in blood samples of 134 cases (51.5% culture positive). (Table2). The commonest organism isolated was Candida spp. (25%). Gram-positive bacteria were isolated in 34 % cases & Gram-negative bacteria in 45 % cases.

Table 1

Clinical Presentation of the study subjects		
Clinical Presentation	No.	Percent
Refusal to feed	145	56.7
Abnormal temperature	101	38.8
Hypothemia	82	31.5
Hyperthermia	18	6.9
Letharginess	98	37.6
Respiratory distress	93	35.7
Apnoea	31	11.9
Diarrhea	21	8.0
Abdominal distention	16	6.1
Vomiting	13	5.0
Seizure	11	4.2

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Table 2

Isolates on blood culture		
Isolates	**No.**	**Percent**
Candida spp.	**32**	**22.5**
Gram-positive isolates	**49**	**34.5**
CONS	27	19.0
Staphylococcus aureus	12	8.4
Enterococcus spp	8	5.6
Micrococci	2	1.4
Gram-negative isolates	**59**	**41.5**
Klebsiella spp.	23	16.2
Enterobacter spp	12	8.4
Citrobacter spp	10	7.0
Acinetobacter spp	5	3.5
Pseudomonas aeruginosa	4	2.8
Escherichia coli	3	2.1
Proteus mirabalis.	2	1.4

Gram-Negative rods were screened and tested for ESBL production. A total of 59 strains of Gram-negative rods were tested for presence of extended-spectrum β- lactamase enzyme (Table 3). Majority of the Klebsiella (56.5%) and Enterobacter (50.0%) isolates were ESBL producing.

Table 3

ESBL production in Gram-negative bacterial isolates

Bacteria Percent	**No of isolates tested For ESBL production**	**No. of isolates producing ESBL**	**%**
Klebsiella	23	12	52.1
Enterobacter	12	5	50.0
Citrobacter	10	3	30.0
Acinetobacter	5	1	20.0
Pseudomonas	4	0	0.0
E. coli	3	0	0.0
Proteus mirabalis.	2	0	0.0
Total	59	21	35.6

Antibiotic resistance pattern of the ESBL producer and non-ESBL producer gram negative organisms was studied. Statistical comparison of resistance patterns of ESBL and non-ESBL producing strains of Klebsiella and Enterobacter has been shown in Table 4 & 5 respectively. The number of other isolates was too small for valid statistical evaluation.

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Table 4

Resistance pattern of Klebsiella spp.

	Isolates (n=23)		ESBL producers (n=12)		Non-ESBL producers (n=11)		
Antibiotic	**No**	**%**	**No**	**%**	**No**	**%**	**p value**
Penicillin	21	91.3	11	91.6	9	81.8	0.47
Cefotaxime	13	56.5	10	83.3	3	27.2	<0.01*
Ceftazidime	10	43.4	8	66.6	2	18.1	0.01*
Cefoperazone +Sulbactam	9	39.1	7	58.3	1	9.0	<0.01*
Amikacin	3	13.0	2	16.6	2	18.1	0.92
Gentamicin	16	69.5	9	75.0	7	63.6	0.63
Ciprofloxacin	4	17.3	3	25.0	2	18.1	0.65

p-value < 0.05 is significant

Table 5

Resistance pattern of Entarobacter spp.

	Isolates(n=12)		ESBL producers (n=5)		Non-ESBL producers (n=7)		
Antibiotic	***No***	***%***	***No***	***%***	***No***	***%***	***p value***
Penicillin	10	83.3	4	80.0	6	85.7	0.64
Cefotaxime	7	58.3	4	80.0	3	42.8	0.03*
Ceftazidime	6	50.0	3	60.0	2	28.5	0.12
Cefoperazone+Sulb	5	41.6	4	80.0	1	14.2	0.01*
Amikacin	3	25.0	1	20.0	1	14.2	0.48
Gentamicin	10	83.3	4	80.0	6	85.7	0.86
Ciprofloxacin	1	8.3	1	20.0	1	14.2	0.48

p-value < 0.05 is significant

Resistance to penicillin and gentamicin was very obvious for both bacteria. Resistance to cephalosporins was observed in higher number in ESBL producing Klebsiella and Enterobacter compared to non-ESBL producers. The difference was significantly high for all cephalosporins tested for Klebsiella and cefotaxime and cefoperazone+sulbactum for Enterobacter. Resistance to amikacin and ciprofloxacin was less in either group for both bacteria. 5 of 59 cases left the hospital against medical advice. Thus, outcome for 54 neonates was available for analysis. It was observed that 20.3% neonates expired (Table 6). The Mortality ratio was significantly higher in ESBL Producer group than in non-ESBL producers.

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.**Table 6**

Mortality in relation to ESBL Production

Isolates	No. of cases	Expired	
		No.	%
ESBL producers	23	8	34.8
Non-ESBL Producers	31	3	9.7
Total	54	11	20.3

Discussion

Despite major improvement in neonatal care and increased survival of newborns over the past three decades, infection still continues to be a major problem for physicians taking care of newborn babies. Clinical diagnosis of an infection is difficult in neonates as the signs are usually subtle and nonspecific. Sepsis definition used for pediatric patients usually do not apply to neonates, due to the fact that they rarely get febrile, and their vital signs are affected by several reasons other than infection such as cold, asphyxia, stress hypoglycemia, and pain etc. Therefore, sepsis is usually suspected in a newborn just based on poor feeding or a state of not being well observed by caregiver or sometime just based on major or minor risk factors found in history.[7] The common clinical features in our study are similar as those observed by others.8,9,10 The absence of well defined criteria and laboratory methods for the diagnosis of sepsis gives rise to inappropriate antibiotic usage which later on results in antibiotic resistance and fungal infection.11 The microbiological spectrum of neonatal septicemia shows marked geographical variations. It may range from acquired from mother during perinatal period to community acquired ot hospital acquired. These organisms are usually resistant strains of the pseudomonas spp. enterobacteriacae, and staphylococci. The overall incidence of septicemia confirmed by blood culture in our study is 54.6%. Reports from India and other countries show a variable incidence ranging from 36% to 55%.12,13,14 The commonest organism isolated was Candida spp. (22.5%). A slightly higher incidence of Candidal septicemia (34.7%) was reported by Rani et al.15 Inappropriate use of antibiotics and poor asepsis maintenance in neonatal units usually results in high incidence of candidal infections.

The most frequent Gram-positive isolate in our study was Coagulase Negative Staphylococci (CONS) (19.1%). A slightly lower 16.6% incidence of CONS septicemia has been reported by Amita Jain et al.9 Freeman et al. (1987) reported a rise from 25 to 68.8% of CONS septicemia in neonates between 1975-1982 at their places, which is an alarming situation.16 In the present study, the most frequent Gram negative bacterial isolates are Klebsiella spp. (16.2%), in accordance with other Indian studies.14,17 The prevalence of Entrobacter sepsis is alarming; a report from Pakistan in 1996 expressed concern about increasing Enterobacter infection in neonates.18 The high percentage of ESBL-producing organisms may be due to selective growth promoted by extensive use of antibiotics in the NICU. Some earlier reports demonstrated a lower prevalence of ESBLproducing bacteria, compared to that of present study.19,20 A study done in central India reported that 76.5% of Klebsiella isolates which were resistant to third-generation cephalosporin were ESBL producers.21 Another study from South India reported an incidence of 58.06% for ESBL producing

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E. Coli, and 57.14% for ESBL-producing Enterobacter spp.22

In our study as far as the routine susceptibility test is concerned; the majority of the resistant isolates were ESBLproducers. While these strains remain susceptible to ceftazidime or cefotaxime in vitro, there is decreased possibility that these drugs are effective in treating infections, caused by ESBL-producing organisms unless the infection is limited to the urinary tract.23 Almost all ESBL producing organisms were resistant to pencillin. One study reported that ciprofloxacin resistance and ESBL production in Klebsiella pneumoniae were closely related.24 They found that that, globally, 18% of ESBL producers were resistant to ciprofloxacin. Our study also shows similar result. The cost of antibiotics has always been a limiting factor in therapy planning; keeping this in mind, it is worth nothing that in comparison to other antibiotics, resistance to amikacin and ciprofloxacin was less frequent. A recent study has found ciprofloxacin to be highly effective in treating multiresistant Gram negative infections, including use in premature and extremely low birth weight infants.[25]In our region,ESBL production testing is not routinely done by most pathologies, This may facilitate the spread of ESBL-producing bacteria in hospital form one patient to other and remain undetected for long periods. The consequence can be serious outbreaks, particularly in the intensive-care units. The mortality in neonates with septicemia was high (20.3%) in our NICU. It was even higher in cases of Gram-negative septicemia; particularly the cases from which ESBL producing organisms were isolated. These patients showed discouraging results with the antibiotic therapy. With the fact that there is high prevalence of ESBL-producing bacteria in our NICU, we feel it is extremely important to revise our antibiotic policy and regular monitoring of ESBL producing organisms in NICU isolates.

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