Prevalence of Extended-Spectrum β-Lactamase Mediated Resistance in Neonatal Septicemia

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Abstract

With the emergence of Extended Spectrum Beta Lactamase (ESBL) producing Gram negative bacilli (GNB) as the predominant pathogen from cases of neonatal septicemia, this study was specially designed to investigate the prevalence of ESBL producing GNB in neonatal septicemia and to compare the antibiotic susceptibility pattern of ESBL producers and non-producers. Total of 1190 blood samples were studied. Gram negative isolates were screened for ESBL production by using disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. Phenotypic confirmatory test was carried out for all the screened ESBL positive isolates by double disc synergy method and combined disc diffusion method. Out of 1190 blood cultures 235 samples showed Gram negative bacterial isolates. All the Gram negative bacilli were screened for ESBL production. Out of 110 probable ESBL producers 82 isolates were found to be confirmed ESBL producers. In our study, ESBL production coexisted with resistance to β-lactam as well as non β-lactam antibiotics like quinolones, aminoglycosides etc. Considering the grave nature of antibiotic resistance in India, it is necessary to ascertain the prevalence of ESBL positive strains in a hospital. This in turn will help in guiding appropriate antibiotic use.

Key words : *Neonatal septicemia, Neonatal Intensive Care Unit (NICU), Extended Spectrum Beta Lactamase (ESBL), Double Disc Synergy Test (DDST), Combined Disc Diffusion Test (CDDT).*

Introduction

Blood stream infections are life threatening and have very poor prognosis, specially infections among neonates admitted to the Neonatal Intensive Care Unit (NICU). This is considered to be one of the leading causes of neonatal morbidity and mortality. The incidence of neonatal sepsis according to data from the National Neonatal Perinatal Database (NNPD, 2002–03) is 30 per 100 live births, contributing to 19% of all neonatal deaths.[1,2]

With the emergence of Extended-Spectrum Beta Lactamase (ESBL) producing gram negative bacilli as the predominant pathogen responsible for Neonatal Septicemia,[3] the third generation cephalosporins, which have been used extensively as a life saving first-line antibiotic among septicemic neonates are rendered useless.[4] ESBL-producing organisms also contain resistance determinants to other important antibiotic groups such as aminoglycosides and fluoroquinolones, hereby limiting therapeutic options.[5] Delay in managing infections with ESBL producers is associated with increased mortality and prolonged hospital stay.

It is therefore necessary that ESBL detection should routinely be carried out. Unfortunately most laboratories in India do not routinely test for ESBL production. [6]

Keeping these facts in mind, this study was specially designed to investigate the prevalence of ESBL producing gram negative bacilli (GNB) in neonatal septicemia and to make medical practitioners aware of the magnitude of the problem.

Aims and Objectives

1. Analyzing blood cultures from NICU patients.
2. Detecting ESBL production among gram negative bacill. GNBs and to compare the antibiotic susceptibility patterns of ESBL producers and non-producers.
3. Studying results of two confirmatory tests for ESBL detection:
   a) Double Disc Synergy Test (DDST)
   b) Combined Disc Diffusion Test (CDDT).

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Materials and Methods

The present study was conducted over a period of two years, from January 2009 to December 2010. During this period a total of 1190 blood samples, consecutively collected from suspected cases of neonatal septicemia admitted to the NICU of a tertiary care hospital were studied in the Department of Microbiology. Samples from older children /adults and from neonates with other clinical diagnosis were excluded. All the samples were collected before administration of antibiotics and processed by standard methods. Blood (1-2 ml) was collected in 10 ml of brain heart infusion broth (Hi-media laboratories, Mumbai, India) with 0.05 percent sodium polyanethol sulphonate. The broth was incubated overnight at 37°C. A subculture was carried out on McConkey agar plate and blood agar plate (Hi-media laboratories, India). If no growth was obtained, the bottles were incubated for seven days and examined on alternate days for evidence of bacterial growth. Any sign of growth was followed by subculture and identification by Gram staining and relevant standard biochemical tests.[7, 8]

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was determined by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standards Institute-2006 (CLSI) recommendations. The drugs tested were ampicillin (10µg), amoxycillin /clavulanic acid (20/10µg), ciprofloxacin (5µg), piperacillin (100µg), piperacillin/ tazobactam (100/10µg), ticarcillin/ clavulanic acid (75/ 10µg), cephotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), cefpodoxime (10µg), gentamicin (10µg), amikacin (30µg), netilmicin (30µg), tetracycline (30µg), chloramphenicol (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg) and imipenem (10µg). *E. coli* ATCC25922 and *K. pneumoniae* ATCC 700603 were used as control strains.

Screening for ESBL:

Isolates were screened for ESBL production by using disk diffusion for cefotaxime, ceftazidime, ceftriaxone and cefpodoxime placed on inoculated plates containing Muller Hinton agar according to CLSI recommendations. Isolates showing inhibition zone size of ≥22mm with ceftazidime (30µg), ≥25mm with ceftriaxone (30µg), ≥27mm with cefotaxime(30µg) and ≥ 17 mm for cefpodoxime were screened as potential ESBL producers. *E. coli* ATCC 25922 was used as a negative control and *K.pneumoniae* ATCC 700603 was used as positive control for ESBL production.

Confirmatory test for ESBL: Phenotypic confirmatory test for ESBL producers was carried out for all isolates that were screened positive for ESBL production by double disc synergy method as described by Jarlier et al[9] and combined disc diffusion method following CLSI guidelines.

Double disk approximation or double disk synergy method: In this test a disk of third-generation cephalosporin was placed 30 mm from a disk of Amoxicillin-Clavulanic acid. Increase in inhibition zone diameter towards combination disks with Clavulanic acid indicated ESBL.[9]

Combined disk diffusion method: In this test a disk of ceftazidime (30µg) alone and a disk of ceftazidime in combination with clavulanic acid (30/10µg) was used. Both the disks were placed 25mm apart, centre to center, on a lawn culture of the test isolate on Muller Hinton agar plate and incubated overnight at 37°C. Difference in zone diameter with and without clavulanic acid was measured. A positive result was defined as a ≥5mm increase in inhibition zone diameter around combination discs with clavulanic acid versus its standard zone when tested alone.[10]

Statistical Analysis:-

Statistical analysis was carried out using SPSS17 to find "standard error of difference between two proportions" (Z test). The test was used to ascertain the significance of difference between the resistance levels of various drugs in ESBL producer strains and non-ESBL producer strains.

Results

Total 1190 blood cultures from NICU neonates were studied over a period of 2 years. Of these 298 samples were positive for bacterial isolates. Out of 298 samples, 235 samples showed gram negative bacterial isolates. Rest of the samples (63) showed Gram positive cocci (n=60) and Candida species (n=3).

Table 1: Spectrum of Gram negative bacterial isolates

Organism Isolated	No. of Isolates	% of Isolates
K. pneumoniae	101	42.9
E. coli	63	26.8
Acinetobacter species	26	11.6
Pseudomonas species	24	10.2
Proteus species	05	2.12
Enterobacter species	16	6.80

All the Gram negative bacilli (n=235) were screened for ESBL production and 110 (47 %) of these isolates were probable ESBL producers. Out of 110 probable ESBL producers, 82 isolates were found to be confirmed ESBL producers.

Among the confirmed 82 ESBL producers 76 isolates were confirmed by CDDT and 70 isolates were confirmed by DDST, whereas 65 isolates showed positive results by both the phenotypic methods.

Table 2: Results of screening and confirmatory tests (CDDT & DDST) for ESBL production

Organism	No.of probable ESBL producers (n=110)	No.of confirmed ESBL producers (n=82)	No of isolates confirmed by CDDT only (n=76)	No of isolates confirmed by DDST only (n=70)	No of isolates confirmed by Both (n=64)	No of isolate1s negative by both(n=28)
K. pneumoniae	40	36	34	33	31	04
E. coli	30	22	21	21	20	08
Acinetobacter species	12	07	06	05	04	05
Pseudomonas species	14	10	09	07	06	04
Proteus species	04	01	01	00	00	03
Enterobacter species	10	06	05	04	03	04

CDDT: Combined Disc Diffusion Test, DDST: Double Disc Synergy Test, ESBL: Extended-Spectrum β Lactamase.

Table 3: Comparison of resistance to antibiotics among ESBL producers and non-producers

Antibiotic	Resistance in SBL Producers (%)	Resistance in Non-ESBL Producers (%)
Ampicillin (10μg)	79 (96.34)	52 (33.98)
Ciprofloxacin (5μg)	78 (95.12)*	48 (31.37)
Piperacillin (100μg)	37 (45.12)	13 (8.49)
Piperacillin/tazobactam (100/10μg)	4 (4.87)	4 (2.61)
Ticarcillin/ clavulanic acid (75/10μg)	5(6.09)	4 (2.61)
Cephotaxime (30μg)	78 (95.12)	5 (3.26)
Ceftriaxone (30μg)	76 (92.68)	9 (5.88)
Ceftazidime (30μg)	80 (97.56)	7 (4.57)
Cefpodoxime (10μg)	78 (95.12)	2 (1.30)
Gentamicin (10μg)	64 (78.04)*	21 (13.72)
Amikacin (30μg)	56 (68.29)*	17 (11.11)
Netilmicin (30μg)	62 (75.60)*	12 (7.84)
Tetracycline (30μg)	54 (65.85)*	32 (20.91)
Cephepime (30μg)	00	1 (0.65)
Chloramphenicol (30μg)	46 (56.09)*	40 (26.14)
Trimethoprim- sulfamethoxazole (1.25/23.75μg)	50 (60.97)*	55 (35.94)
Imipenem (10μg)	1 (1.21)	3 (1.96)

* P< 0.05 i.e. ESBL producer strains were significantly more resistant than non-ESBL producer strains.

Comparison and analysis of antibiotic sensitivity patterns of ESBL producers and Non-producers (Table 3) showed that ESBL producers exhibit significantly more resistance to β-lactam as well as non β-lactam antibiotics than non-ESBL producers.

Discussion

Neonatal septicemia remains a major clinical problem in neonatology with high morbidity and mortality.[11] For over a decade now, third generation cephalosporins, especially cefotaxime and ceftazidime along with gentamicin are used as preliminary therapy for clinically suspected cases of septicemia.[6] With emergence of ESBLs, the third generation cephalosporins which are used extensively as a life saving first line antibiotic among septicemic neonates are rendered useless.

In the present study, out of 298 bacterial isolates, most were gram negative bacilli (79%), of which 82 (35%) were ESBL producers. These findings are similar to studies conducted by A. Bhattacharjee et. al, [12] who reported 32% ESBL producers out of 73.04% of GNB isolates from NICU patients. This is contrary to study conducted by Kumar et. al, [13] who reported 13.54% and a much lower figure than reported by Jain A., et. al. (86.6 %).[5] It was also observed that prevalence of ESBL varies with geographical locations within the same country.[12] The incidence of ESBL reported by Vinod Kumar et. al[14] in 2006 was 22.7%. Sehgal et. al[15] in 2007 reported ESBL incidence as 61.3%. Although, frequency of ESBL producing *E. coli* in Europe, North Latin America and Western Pacific is reported between 1-8%, its prevalence in the Asia Pacific region and South Africa is reported at more than 20%.[16]

A total of 110 isolates out of 236 screened were presumptively considered as ESBL positive on the basis of their resistance to the four screening agents. The predictive value was highest with cefpodoxime and least with ceftriaxone. This is in accordance with the finding of Rao et al 2008.[17]

Out of 82 confirmed ESBL producing isolates, 76 isolates were positive by CDDT method only, 70 were positive by DDST only and 64 were positive by both methods. Thus out of 82 isolates CDDT detected 92.6% and DDST detected 85.3% of cases.

In our study, ESBL production coexisted with resistance to β-lactam as well as non β-lactam antibiotics like quinolones, aminoglycosides etc. Among the ESBL producers, 96.34% isolates showed resistance to ampicillin, 60.9% resistance to cotrimoxazole, 65.85% resistance to tetracycline, 56.09% resistance to chloramphenicol, 78.04 % resistance to gentamicin and 95.12% resistance to ciprofloxacin. Such a wide resistance spectra of ESBL producers have been observed by many others. [3, 18, 19]

On review of third generation cephalosporin resistance patterns of the isolates, we found that among the ESBL producers, 95.12% were resistant to cefotaxime, 92.68% to ceftriaxone, 97.12% to ceftazidime and 95.12% to cefpodoxime. As ceftriaxone was found to be the most common cephalosporin administered to the neonates in our NICU, it is recommended that ESBL screening should be made mandatory, particularly when using ceftriaxone as a prime screening agent.

A combination of β-lactam and β-lactamase inhibitor, particularly piperacillin/tazobactam and ticarcillin-clavulanic acid, showed greater activity in both groups (ESBL producers and non-producers).

Among Aminoglycosides, amikacin showed greater sensitivity to all isolates irrespective of ESBL production status which is comparable to other studies.[20,21]

All the isolates were found to be sensitive to carbapenems except Pseudomonas species in which 1.21% of the isolates from ESBL producers and 1.96% isolates from Non-ESBL producers showed resistance to Imipenem.

As increased number of MBL producers are being found to be resistant to carbapenems, screening for MBLs should also be implemented along with screening for ESBLs.

Conclusion

Considering the serious nature of antibiotic resistance in India, it is necessary to determine the prevalence of ESBL positive strains in a hospital so as to formulate a policy of empirical therapy in high-risk units like NICUs. Equally important is the information on an isolate from a patient, which will help in appropriate antibiotic use with an improved outcome. It is possibe that the restricted use of antibiotics can lead to withdrawal of selective pressure and resistant bacteria may no longer have survival advantages in a hospital setting.

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