

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.510.087>

## ESBL Producing Gram Negative Bacteria-A Cause of Concern in Neonatal Septicemia in a Tertiary Care Hospital

Ashish Khanna<sup>1\*</sup>, Menka Khanna<sup>2</sup> and Manmeet Gill<sup>1</sup>

<sup>1</sup>Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Punjab, India

<sup>2</sup>Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and Research, Punjab, India

\*Corresponding author

### ABSTRACT

Neonatal septicaemia is the leading cause of death in neonates admitted in the neonatal intensive care unit in India. Furthermore this situation is nowadays worsened by the beta lactamase producing bacteria occurring as a causative agent of neonatal septicaemia. These ESBL producing gram negative organisms with increased resistance should be monitored regularly and antibiogram prepared so as to plan effective empiric therapy. Aim of this study was to determine the bacteriological spectrum and resistance pattern of these gram negative isolates from neonatal intensive care unit. ESBL production among these gram negative organisms was also determined. A study was done in the department of Microbiology of our medical college where 282 neonates admitted in the neonatal intensive care unit between June 2016 to August 2016 with suspicion of septicaemia were included in the study. About 2ml of blood was collected under aseptic condition and put in Bact T Alert PF plus bottles (paediatric bottles) for culture. Gram negative isolates recovered from these septicemic patients were further tested for the presence of ESBL and resistance pattern. Among the 282 suspected patients of neonatal septicaemia, blood culture was positive in 89 patients. Gram negative isolates were seen in 48 cases whereas gram positive isolates were seen in 22 cases. *Candida* species were isolated from 19 cases of neonatal septicaemia. Most common gram negative organism isolated was *Klebsiella* species (22.2%). ESBL production was there in 39.6% of the gram negative isolates. Multidrug resistance was more common in ESBL producers than Non ESBL producers. Septicaemia in neonates is increasing rapidly in India. ESBL producing gram negative organisms are also increasing with alarming proportion throughout the world. As the gold standard for the diagnosis of septicemia is culture which takes about 48-72 hours so precious time is lost in the diagnosis. Clinician has to rely on the empirical therapy till the result of the culture is obtained. Empiric therapy should be based on the knowledge of prevalence of beta lactamases in that hospital. Bacteriological profile of neonatal septicaemia also varies in different regions of the vast country like India.

#### Keywords

ESBL, Neonatal septicemia, MBL, Gram negative bacteria, NICU.

#### Article Info

Accepted:  
25 September 2016  
Available Online:  
10 October 2016

## Introduction

Neonatal septicemia refers to a generalized bacterial infection documented by positive blood culture in first four weeks of life (Gottof *et al.*, 1970). In recent times there have been a lot of advances in antimicrobial treatment, neonatal life supports as well as early detection of risk factors. Septicemia continues to be to a major cause of neonatal mortality and morbidity and is responsible for 30 – 50% of total neonatal deaths (Tripathi *et al.*, 2010). Dependig upon the age of onset neonatal septicemia manifests in 2 forms-early onset septicemia (EONS) and late onset septicemia (LOS). EONS is defined as a positive result in one or more blood culture within 72 hours of life together with clinical signs and symptoms of sepsis. Late onset septicemia, when the same occurs after 72hrs of life (Prashar *et al.*, 2001). Maternal, host and environmental factors determine which infant exposed to potentially pathogenic organism will develop sepsis. The etiological agents implicated in causation of neonatal septicemia vary from place to place and also change with time and antibiotic usage (Lodha *et al.*, 2001). Of particular concern is the increasing resistance to routine antibiotics among pathogens with resistant organisms more frequently found in hospital environment secondary to misuse of antibiotics. The most prevalent mechanism of drug resistance at this time is considered to be the production of beta lactamase enzyme. The overall prevalence of beta lactamases and extended spectrum beta lactamases is rising with increased use of higher generation antibiotics (Sirot, 1995).

Therefore a rational protocol for sepsis management must be based on adequate knowledge of the causative organism and their antibiotic sensitivity pattern .Also the bacteriological profile of neonatal sepsis is constantly under change with advances in

early diagnosis and treatment. Keeping these points in mind the present study was undertaken to know the bacteriological spectrum along with antibiotic sensitivity pattern of blood culture isolates from the suspected cases of neonatal septicemia.

## Materials and Methods

About 282 neonates admitted in the neonatal intensive care unit of Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar between june 2016 to august 2016 with suspicion of septicaemia were included in the study. About 2ml of blood was collected under aseptic condition and put in Bact T Alert PF plus bottles (paediatric bottles). These bottles were then put in Bact T Alert blood culture machine by Biomerieux. The machine works on calorimetric technology. The bottles contained antimicrobial neutralization resins which helps to minimize the false negative results.

Bottles showing positive cultures were subcultured on blood and macconkey agar and put for overnight incubation at 37<sup>o</sup>C.Growth obtained was identified by gram staining, culture characters as well as routine biochemical reactions. The resistance pattern of the gram negative bacteria was determined by putting up antibiotic sensitivity testing by disc diffusion method (Kirby Baur method). Mueller Hinton agar (HiMedia) was used for doing the susceptibility testing by the disc diffusion method as per CLSI guidelines (Wayne, 2010). The following antibiotics of standard potency were used: ceftazidime, cefotaxime, cefepime, cefoxitin, ceftriaxone, and imipenem, Piperacillin, amikacin, netilmicin, gentamicin, ciprofloxacin, piperacillin/ tazobactam, ticarcillin/clavulanic acid. All gram negative isolates having reduced sensitivity to

ceftazidime (zone diameter of <22 mm), ceftriaxone (zone diameter of <25 mm) or cefotaxime as per CLSI guidelines were further confirmed for the ESBL production by double-disc diffusion method and double disc synergy test by using *E. coli* ATCC 25922 as control.

After swabbing the isolates on muller hinton plate disc of ceftazidime and ceftazidime + clavulanic were placed apart. The plates were incubated at 37°C for 24 hours. After incubation >5mm difference in the inhibition zone of ceftazidime + clavulanic acid disc as compared to ceftazidime disc alone was taken as ESBL positive. This was further confirmed by Triple ESBL Ezy MIC strip (himedia). This E strip contains a mixture of three different antibiotic with or without inhibitors like clavulanic acid in a concentration gradient. The strains having ratio of > 8 when the MIC of mixture plus and mixture is compared respectively are taken as ESBL positive. Further resistance in ESBL producing gram negative bacteria was compared with resistance in non ESBL producers.

All isolates were further tested for MBL production with the disc of imipenem and imipenem in combination with EDTA placed at a distance of 20 mm apart on a muller hinton plate which was incubated overnight at 37°C. Difference in inhibition zone of > 5 mm between the two was taken as positive for MBL production. *P. aeruginosa* ATCC 27853 was used as a negative control strain (Shahid *et al.*, 2004; Yong *et al.*, 2000).

## Results and Discussion

Among the 282 suspected patients of neonatal septicaemia, blood culture was positive in 89 (31.5%) patients. Bacteriological profile of the culture positive patients is given in table 1. Gram

negative isolates were seen in 48 (53.9 %) cases whereas gram positive isolates were seen in 22 (24.7%) cases. *Candida* species were isolated from 19 cases of neonatal septicaemia. Most common organism isolated was *Klebsiella* species (22.2%) followed by *Candida* species (21.3%) among all the isolates from patients of neonatal septicaemia. *Staphylococcus aureus* (15.7%) and *E.coli* (14.6%) were the other common organisms recovered from neonatal septicemic patients. ESBL production was seen in 19(39.6%) of the 48 gram negative isolates. ESBL production was mostly seen in *Klebsiella* (55.5%) and *E.coli* (46.2%) group of organisms among the family enterobacteriaceae. Table 2 Among the non fermenters high ESBL production was seen in *Acinetobacter* species (33.3%). Mutidrug resistance was seen in most of the ESBL producing strains which was higher than that seen in non ESBL producing strains. Table 3 MBL production was seen in five isolates only (two in *Klebsiella* and *Acinetobacter* species and one in *pseudomonas*). These beta lactamase producers were further tested for second line of antibiotics like colistin, polymyxin B and tigecycline.

Neonatal septicaemia is estimated to cause about 5 million neonatal death every year world wide.<sup>10</sup> Multiple antibiotic resistances among neonatal sepsis are currently one of the greatest challenges to the effective management of infections. Out of blood samples, septicemia could be confirmed by culture in 31.5% (89 out of 282) cases. There has been a wide variation in the growth positivity obtained by blood culture over the years. A higher isolation rate of 52.6% and 47.5% was reported by Murty *et al* and Rajendraprasad *et al* respectively (Murty *et al.*, 2007).

Out of 282 cases, 122 cases (43.3 %) were of EOS and 160 cases (56.7 %) were of LOS. This clustering of 43.3% cases in first

3 days of life reflects the immaturity of immunological responses in the first few days of life. The EOS occurs due to ascending infection from infected birth canal or following rupture of membrane usually caused by Gram-negative organisms acquired after birth from human contact. Movahedian *et al.*, have reported 81.5% cases of early onset neonatal septicemia.

In the present study, Gram-negative organisms predominated being responsible for (48 out of 89) 53.9 % of cases of septicaemia in which culture was positive. A recent study conducted in Karnataka reported 70.5% neonatal septicemia cases caused by Gram-negative isolates. Among

all the gram negative isolates *Klebsiella* species has been found to be predominant pathogen (20.2 %) followed by *Escherichia coli* (14.6 %). This predominance of *Klebsiella* species was correlated with the study done by Mathur *et al.*, (1994).

A total 24.7% of Gram-positive organisms have been observed in our study, similar kind of results also find in Roy *et al.*, (2002). Amongst the Gram-positive organisms *Staphylococcus aureus* was the predominant pathogens (15.7%). The results of antibiotic sensitivity pattern revealed that majority of Gram-negative organisms were resistant to commonly used antibiotics like amoxicillin, penicillin and cephalosporins.

**Table.1** Showing distribution of various organisms in patients of neonatal septicaemia.(n=89)

Organism	number	Percentage
<i>Klebsiella</i>	18	20.2
<i>E.coli</i>	13	14.6
Enterobacter	1	1.1
Citrobacter	1	1.1
Proteus	2	2.2
<i>Acinetobacter</i>	6	6.7
Pseudomonas	7	7.9
Staphylococcus Aureus	14	15.7
Coagulase negative Staph	6	6.7
Streptococcus sp	1	1.1
Candida species	19	21.3

**Table.2** Showing ESBL production by gram negative organisms

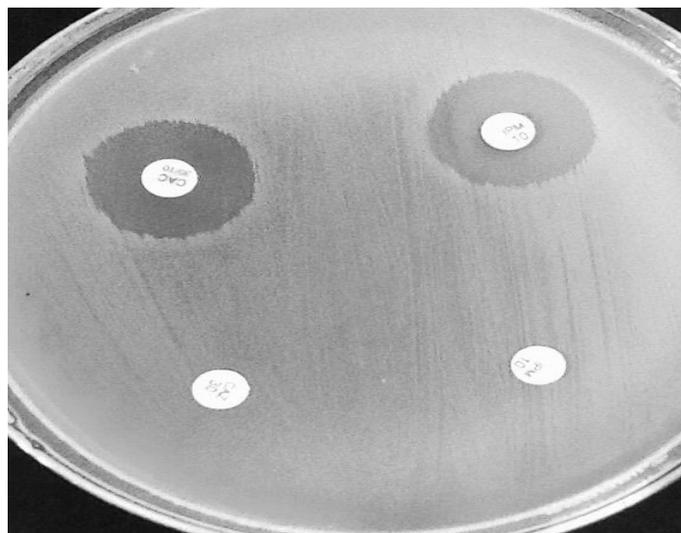
Organism	Total no of isolates	No of isolates producing ESBL(%)
<i>Klebsiella</i>	18	10(55.5)
<i>E.coli</i>	13	6(46.2)
Enterobacter	1	-
Citrobacter	1	-
Proteus	2	-
<i>Acinetobacter</i>	6	2(33.3)
Pseudomonas	7	1(14.3)
Total	48	19(39.6)

**Table.3** Resistance pattern among ESBL producing and Non ESBL producing Gram negative bacteria (number of isolates showing resistance)

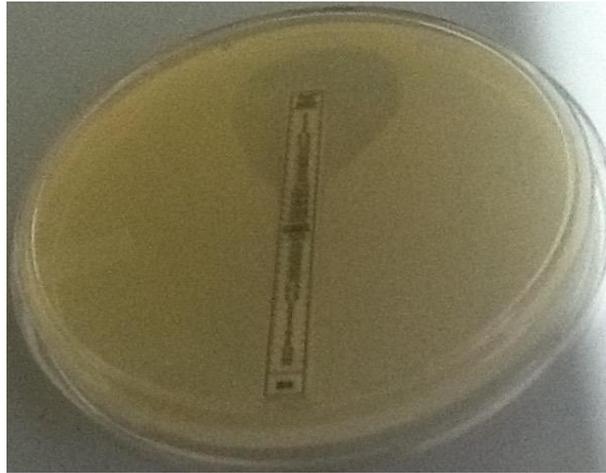
Antibiotic	Organism							
	<i>Klebsiella</i> (n=18)		<i>E.coli</i> (n=13)		<i>Acinetobacter</i> (n=6)		<i>Pseudomonas</i> (n=7)	
	ESBL 10	NoESBL 8	ESBL 6	NoESBL 7	ESBL 2	NoESBL 4	ESBL 1	NoESBL 6
Amikacin	9	5	5	3	2	2	-	2
Gentamycin	9	3	6	2	2	3	1	4
Piperacillin	8	2	4	2	2	3	-	1
Cefotaxime	8	4	5	3	2	3	1	2
Ceftriaxone	5	2	6	4	2	2	1	3
Ceftazidime	7	3	4	1	2	3	1	2
Cefipime	4	2	2	2	1	1	-	1
Cefoxitin	6	2	4	2	2	3	1	2
P/T	3	1	1	-	2	3	-	-
T/C	4	2	1	-	2	3	-	-
Imipenem	2	1	2	-	1	1	1	-

P/T-Piperacillin/Tazobactam, T/C-Ticarcillin/Clavulanic acid

**Fig.1** Showing ESBL and MBL production by gram negative isolate (using disc of ceftazidime and ceftazidime/clavulanic acid for ESBL production and Imipenem and Imipenem/EDTA disc for MBL production)



**Fig.2** ESBL production by E strip method



It has been shown that piperacillin/tazobactam and Imipenem were the two most effective antibiotics against Gram-negative organisms. More resistance was seen in ESBL producers than non ESBL producers Table 3. Majority of *S.aureus* was resistant to amoxicillin, quinolones etc but vancomycin still remains the most sensitive drug for *S.aureus*, not a single case of resistant was found against vancomycin, which correlate with the findings of Roy *et al.*, (2015).

The incidence of ESBL and MBL producing strains among gram negative isolates has been increasing alarmingly leading to limited therapeutic alternatives. These enzymes are either plasmid or chromosomally mediated and can be easily transferred to other bacteria lacking them. Previously it was reported by various researchers that these enzymes were mostly seen in *E coli* and *Klebsiella* species. However in recent times there have been reports of these enzymes being produced by all bacteria of family Enterobacteriaceae as well as other gram negative bacteria. 53.9% of the organisms isolated after blood culture in neonatal septicemic patients were gram negative bacteria (48 out of 89). Among these gram negative organisms ESBL

production was seen in 19 (39.6%). Similarly in an earlier study done in north India among neonatal septicemic patients gram negative isolates were seen in 41.5% of the culture positive cases whereas 21 out of 59 gram negative isolates showed ESBL production. These findings are comparable with the finding in our study (Sharma *et al.*, 2015). MBL production was seen in five gram negative organisms isolated from patients of neonatal septicaemia. The only option left for the treatment of these cases was the reserve drugs like colistin, polymyxin B and tigecycline etc. *Candida* species was the commonest organism isolated in our study (19 out of 89 ie 21.3%). Higher incidence of candida infection was also seen in other studies also (Rani *et al.*, 2002). This could be due to use of high end antibiotics in neonatal septicaemia in our tertiary care hospital.

Testing for ESBL is not done in routine laboratories in most hospitals in our country. This leads to alarming spread of ESBL producing organisms in our country particularly in intensive care units. So the empirical therapy in neonatal intensive care units should be regularly adjusted based on the prevalence of ESBL producing organisms in that hospital.

## References

- Collee, J.G., Miles, R.S., Wan, B. 1996. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, eds. Mackie and Mc Cartney Practical Medical Microbiology. 14<sup>th</sup> ed. Edinburgh: Churchill Livingstone; 131-50.
- Gottof, S.P., Behrman, R.E. 1970. Neonatal septicaemia. *Indian J. Pediatrics*, 76: 142-53.
- Lodha, R., Chandra, V., Natchu, M., Nanda, M., Kabra, S.K. 2001. Nosocomial infections in pediatrics intensive care units. *Indian J. Pediatrics*, 68(11): 1063-70.
- Mathur, M., Shah, H., Dixit, K., Khambadkone, S., Chakrapani, A., Irani, S. 1994. Bacteriological profile of neonatal septicemia cases. *J. Postgrad. Med.*, 40: 18-20.
- Mathur, P., Kapil, A., Das, B., Dhawan, B. 2000. Prevalence of extended spectrum beta lactamase producing gram negative bacteria in a tertiary care hospital. *Indian J. Med. Res.*, 115: 153-7.
- Movahedian, A.H., Moniri, R., Mosayebi, Z. 2006. Bacterial culture of neonatal sepsis. *Iranian J. Pub Health*, 33: 84-9.
- Murty, D.S., Gyaneshwari, M. 2007. Blood cultures in pediatric patients: A study of clinical impact. *Indian J. Med. Microbiol.*, 25: 220-4.
- Nalini, A., Neelam, K. and Varsha, G. 2004. Antimicrobial susceptibility of isolates from neonatal septicaemia. *Jpn. Infect. Dis.*, 57: 273-5.
- Prashar, A., Kumar, A. 2001. Clinical approach to neonatal sepsis. *Pediatrics today*, 4(1): 493-8.
- Rajendraprasad, B.P., Basavaraj, K.N., Antony, B. 2013. Bacterial spectrum of neonatal septicemia with their antibiogram with reference to various predisposing factors in a tertiary care hospital in Southern India. *Ann Trop Med Public Health*, 6: 96-9.
- Rani, R., Mahapatra, N.P., Mehta, G., Randhawa, V.S. 2002. Changing trend of neonatal septicemia in a tertiary north Indian hospital. *Ind. J. Med. Microbiol.*, 20(1): 42-4.
- Roy, I., A. Jain, M. Kumar, S.K. Agarwal *et al.* 2002. Bacteriology of neonatal septicemia in a tertiary care hospital of northern India. *Indian J. Med. Microbiol.*, 20(3): 156-59.
- Shahid, S., Malik, A., Agrawal, M., Singhal, S. 2004. The phenotypic detection of the extended spectrum and the AmpC  $\beta$  lactamases by a new spot inoculation method and a modified three dimensional extract test. *J. Antimicrobial. Chemother.*, 54: 684-7.
- Sharma, R.S., Tiwari, M. 2015. Neonatal septicemia in NICU of a tertiary care centre in north India due to extended spectrum beta lact-mase (ESBL ) producing bacteria. *Indian J. Basic and Appl. Med. Res.*, (5):183-90.
- Sirof, D. 1995. Extended spectrum plasmid mediated beta- lactamases. *Antimicrob. Agents Chemother.*, 36: 19-34.
- Tripathi, S., Malik, G.K. 2010. Neonatal sepsis: Past, present and future; a review article. *Internet J. Med. Update*, 5: 45-54.
- Wayne, P.A. 2010. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement M100-S20.
- Yong, D., Lee, K., Yum, J.H., Shin, H.B., Rossolini, G.M., *et al.* 2000. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J. Clin. Microbiol.*, 3798- 3801.

### How to cite this article:

Ashish Khanna, Menka Khanna and Manmeet Gill. 2016. ESBL Producing Gram Negative Bacteria-A Cause of Concern in Neonatal Septicemia in a Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci*. 5(10): 807-813. doi: <http://dx.doi.org/10.20546/ijcmas.2016.510.087>