

Original Research Article

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Prevalence of ESBL in Isolates of *Klebsiella pneumoniae* and *E. coli* in Suspected Cases of Neonatal Septicemia in Tertiary Care Hospital in North Maharashtra, India

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ABSTRACT

Neonatal sepsis is a significant cause of morbidity and mortality in newborns and is responsible for 30 – 50 % of total neonatal deaths. Multidrug resistant Gram negative bacilli belonging to the family *Enterobacteriaceae* have been increasingly responsible for infections among the neonates admitted to the NICU in many countries including India. The study was conducted to know prevalence of ESBL production in *Klebsiella pneumoniae* and *Escherichia coli* in NICU so that we can help clinicians to avoid injudicious use of antibiotics. The present study was carried out from July 2017 to December 2018 in Microbiology Department of the rural medical college in North Maharashtra. A total of 200 samples of blood cultures from neonates admitted in the NICU were processed for the study. 2 ml blood from the neonates was collected under aseptic precautions and the samples were inoculated on blood culture bottle containing Brain Heart Infusion (BHI) broth. Gram negative isolates recovered from septicemic cases were further tested for presence of ESBL and resistance pattern. Blood culture is positive in 134 (67%) among 200 suspected cases of neonatal septicemia. Gram negative isolates are seen in 94 (70.15%) cases whereas Gram positive isolates are seen in 35 (26.11%) cases. The common organism isolated are *Klebsiella pneumoniae* 53 (39.55%) followed by *Escherichia coli* 27 (20.15%) and *Staphylococcus aureus* 25 (18.66%) (Table 1). The ESBL production is more seen in *Klebsiella* 29 / 53 (54.71%) and *Escherichia coli* 12/27 (44.44%). Multidrug resistance is seen in most of the ESBL producing strains which was higher than that seen in no ESBL producing strains. Bacteriological profile of neonatal septicemia varies in different regions of the country like India. Empirical therapy in NICU should be regularly monitored according to the prevalence of ESBL producing organisms in tertiary care centers.

Keywords

ESBL, *Escherichia coli*, *Klebsiella pneumoniae*, Neonatal septicemia, Blood culture

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Introduction

Neonatal sepsis is a significant cause of morbidity and mortality in newborns and is responsible for 30 – 50 % of total neonatal

deaths (Tripathi *et al.*, 2010). The antibiotic susceptibility of microorganisms also changes with time, with the emergence of multidrug resistant organisms (Ballot D *et al.*, 2012). Multidrug resistant Gram negative bacilli

belonging to the family *Enterobacteriaceae* have been increasingly responsible for infections among the neonates admitted to the NICU in many countries including India. *Klebsiella pneumoniae* and *Escherichia coli* constitutes majority of these pathogens (Jain A *et al.*, 2003). With the emergence of ESBL producing *Klebsiella pneumoniae* and *Escherichia coli* as the predominant pathogen, the third generation cephalosporins which have been used extensively as a life saving first line antibiotic among septicemic neonates are rendered useless (Lebessi *et al.*, 2002). Extended spectrum β lactamases (ESBLs) are plasmid mediated, TEM and SHV derived enzymes, first isolated in Western Europe in mid 1980's, most commonly in *Klebsiella spp.*, followed by *E.coli* (Chaudhary U *et al.*, 2004).

The study was conducted to know prevalence of ESBL production in *Klebsiella pneumoniae* and *Escherichia coli* in NICU so that we can help clinicians to avoid injudicious use of antibiotics and giving susceptible antimicrobials to prevent therapeutic failure in neonates having infection with ESBL positive *Klebsiella pneumoniae* and *Escherichia coli*.

Materials and Methods

The present study was carried out from July 2017 to December 2018 in Microbiology Department of the rural medical college in North Maharashtra. A total of 200 samples of blood cultures from neonates admitted in the NICU were processed for the study.

Definitions

Early onset neonatal septicemia (EONS) which is defined as infection occurring in either the first 48 – 72 hrs of life or the first week of life. Late onset sepsis (LONS) is defined as sepsis occurring after 72 hrs or upto 28 weeks of life.

2 ml blood from the neonates was collected by venepuncture under aseptic precautions and the samples were inoculated on blood culture bottle containing Brain Heart Infusion (BHI) broth (Himedia, Mumbai). The bottles were incubated aerobically at 37⁰C for 7 days. The samples were subcultured on blood agar and MacConkey's agar.

The isolates were identified by colony characteristics, Gram staining, motility and standard biochemical tests. Antibiotic sensitivity test was performed by Kirby Bauer disc diffusion method as per Clinical Laboratory and Standard Institute (CLSI) (Wayne P. A., 2010) using Muller Hinton agar plates (MHA) and commercially procured antibiotic discs (Himedia, Mumbai).

Screening and confirmation of ESBLs

Screening for ESBL was done according to the CLSI guidelines. All *Klebsiella pneumoniae* and *Escherichia coli* isolates were screened for ESBL production by Kirby Bauer disc diffusion method. The isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 μ g), ≤ 25 mm with Ceftriaxone (30 μ g), and ≤ 27 mm with Cefotaxime (30 μ g) were identified. Confirmation of ESBL production was done by combined disc diffusion method. This test was done by using a disk of Ceftazidime (30 μ g) alone and a disk of Ceftazidime + Clavulanic acid (30 μ g / 10 μ g) is used. Both discs were placed at 25 mm apart, center to center, on a lawn culture of test isolate on Muller Hinton Agar (MHA) plate and incubated overnight at 37⁰C. A difference in zone diameters with or without clavulanic acid of ≥ 5 mm confirmed ESBL production.

Quality control was done by non – ESBL producing organism (*Escherichia coli* ATCC 25922) and an ESBL producing organism (*Klebsiella pneumoniae* ATCC 700603).

Results and Discussion

Blood culture is positive in 134 (67%) among 200 suspected cases of neonatal septicemia. Gram negative isolates are seen in 94 (70.15%) cases whereas Gram positive isolates were seen in 35 (26.11%) cases. The common organism isolated are *Klebsiella pneumoniae* 53 (39.55%) followed by *Escherichia coli* 27 (20.15%) and *Staphylococcus aureus* 25 (18.66%) (Table 1).

Early onset neonatal septicemia (EONS) is seen in 138 (69%) cases while late onset neonatal septicemia (LONS) is seen in 62 (31%) cases. The distribution of culture positivity in cases of EONS and LONS is given in Table 2.

ESBL production is seen in 41/80 isolates of *Klebsiella pneumoniae* and *Escherichia coli*. The ESBL production is more seen in *Klebsiella* 29 / 53 (54.71%) as compared to *Escherichia coli* 12/27 (44.44%) (Table 3).

Among the nonfermenters high ESBL production is seen in *Acinetobacter* species 2 / 6 (33.33%) and *Pseudomonas aeruginosa* 1 / 8 (12.5%). Multidrug resistance is seen in most of the ESBL producing strains which is higher than that seen in no ESBL producing strains (Table 3).

Neonatal septicemia remains as important cause of morbidity and mortality in developing countries along with developed countries. The accurate and timely identification of etiological agent and their resistance pattern are essential to combat neonatal septicemia. According to other studies, ESBL strains especially *E. coli* and *Klebsiella Species* have frequently implicated in neonatal septicemia at tertiary care center (Krishna *et al.*, 2007, Chandel *et al.*, 2011).

The blood culture positivity rate among neonates in current study was 67%. A wide variation in blood culture positivity has been reported over the years from different centers of our country. This was similar to results of Nandy *et al.*, (2007) and Yashwant Rao *et al.*, (2012) and contrast to results of Rajendraprasad *et al.*, (2013) and Khanna *et al.*, (2016).

In our study, it was found that EONS (69%) was more common than LONS (31%). The EONS may be due to low birth weight, caesarian section, prolonged rupture of membranes (≥ 12 hrs.) and chorioamnionitis as documented in our study. Similar results were observed in Agnihotri *et al.*, (2004).

In the present study, Gram negative isolates were common (70.15%) among all culture positive cases of neonatal septicemia as compared to Gram positive isolates 26.12% and *Candida* species 3.73%. This was in line with results of Movahedian *et al.*, (2007) and Zaki *et al.*, (2009).

In the present study, *Klebsiella pneumoniae* (39.55%) and *E. coli* (20.15%) were common Gram negative organisms isolated from cases of neonatal septicemia. *Staphylococcus aureus* (18.66%) and *Coagulase negative Staphylococcus species* (6.72%) were found to be frequently isolated Gram positive organisms. This was in line with Girish *et al.*, (2012) and Pengsaa *et al.*, (1995).

In the present study, significant no. of ESBL production was seen among *Klebsiella pneumoniae* (54.17%) and *E. coli* (44.44%). This was similar to results of Khanna *et al.*, 2016, Prem Mishra *et al.*, 2018 and Gandhi *et al.*, 2013.

In the present study, 89 – 100% resistance against 3rd generation cephalosporins was observed among ESBL producing strains of

Klebsiella pneumoniae and *E. coli*. Similar results reported by Islam *et al.*, 2014 which reported all ESBL positive strains of *E. coli* were resistant to Cefotaxime, Ceftriaxone and

Ceftazidime as compared to non ESBL isolates (30 – 71%). This was in line with Sharma *et al.*, (2015) (Table 5).

Table.1 Bacteriological profile of Neonatal Septicemia

Organisms	EONS	LONS	Total
Gram positive organisms	24 (26.37%)	11 (25.58%)	35 (26.12%)
<i>Staphylococcus aureus</i>	17 (18.68%)	8 (18.60%)	25 (18.66%)
<i>Coagulase Negative Staphylococcus species</i>	6 (6.60%)	3 (6.98%)	9 (6.72%)
<i>Micrococcus species</i>	1 (1.1%)	0 (0.00%)	1 (0.07%)
Gram negative organisms	64 (70.33%)	30 (69.77%)	94 (70.15%)
<i>Klebsiella pneumoniae</i>	35 (38.46%)	18 (41.86%)	53 (39.55%)
<i>Escherichia coli</i>	21 (23.08%)	6 (13.95%)	27 (20.15%)
<i>Pseudomonas aeruginosa</i>	4 (4.4%)	4 (9.30%)	8 (5.97%)
<i>Acinetobacter species</i>	4 (4.4%)	2 (4.65%)	6 (4.48%)
<i>Candida species</i>	3 (3.3%)	2 (4.65%)	5 (3.73%)
Total	91	43	134

Table.2 Distribution of culture positive cases among EONS & LONS cases

Age of onset of septicemia	Early onset septicemia	Late onset septicemia	Total
Culture Positive	91 (65.94%)	43 (69.35%)	134 (67%)
Culture Negative	47 (34.06%)	19 (30.65%)	66 (33%)
Total	138 (69%)	62 (31%)	200

Table.3 Resistance Pattern among ESBL producing and non producing isolates

Organisms	K. pneumoniae (n = 53)		E. coli (n = 27)		Pseudomonas (n = 8)		Acinetobacter sp. (n = 6)	
	ESBL (n = 29)	Non ESBL (n=26)	ESBL (n =12)	Non ESBL (n = 15)	ESBL (n = 1)	Non ESBL (n = 7)	ESBL (n =2)	Non ESBL (n = 4)
Amikacin	4	5	5	3	0	6	2	2
Gentamycin	21	17	4	4	0	3	2	2
Cefotaxime	26	8	12	5	1	4	2	4
Ceftriaxone	27	9	10	4	1	4	2	3
Ceftazidime	27	8	12	5	1	5	2	4
Cefepime	22	5	9	3	1	2	1	3
Imipenem	7	3	2	3	0	3	1	1
Ciprofloxacin	6	4	3	2	0	2	0	3

Table.4 ESBL production in Gram negative bacterial isolates

Organisms	No. of isolates tested for ESBL production	No. of isolates producing ESBL	Percentage
<i>Klebsiella pneumoniae</i>	53	29	54.71
<i>Escherichia coli</i>	27	12	44.44
<i>Pseudomonas aeruginosa</i>	8	1	12.5
<i>Acinetobacter species</i>	6	2	33.33
TOTAL	94	44	46.80

Imipenem shows 16 – 50%, aminoglycosides like Amikacin 14– 42% and Gentamycin 33 – 72% shows resistance for ESBL producing *Klebsiella pneumoniae* and *E. coli*. This was in line with results of Prem Mishra *et al.*, 2018.

In conclusion, testing for ESBL production is not routinely done by most of tertiary care centers. This may leads to ESBL producing isolates within tertiary care centers particularly in NICU. So empirical therapy in NICU should be regularly monitored according to the prevalence of ESBL producing organisms in tertiary care centers.

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