

## Neonatal sepsis due to ESBL producing *Klebsiella*

Anitha T.K<sup>1</sup>, Anuradha K<sup>2\*</sup>

<sup>1</sup>Assistant Professor, <sup>2</sup>Professor and HOD, Dept. of Microbiology, <sup>1</sup>Kanachur Institute of Medical Sciences, Mangalore, Karnataka, <sup>2</sup>Mysore Medical College and Research Institute, Mysore, Karnataka, India

**\*Corresponding Author: Anuradha K**

Email: drkanuradha@yahoo.com

### Abstract

**Introduction:** Septicemia is a major cause of morbidity and mortality among neonates in India, significantly threatening the survival in this period. Over the past decade, *Klebsiella* species, especially ESBL producers have emerged as an important cause of neonatal septicemia. ESBL producing *Klebsiella* species are of serious concern as they exhibit high resistance to commonly used antibiotics. Hence, the present study was undertaken to evaluate the prevalence of ESBL producing *Klebsiella* species as a causative agent in neonatal sepsis.

**Objectives:** 1. To determine the incidence of *Klebsiella* species as an etiological agent of neonatal septicemia from blood cultures in patients admitted in Neonatal Intensive Care Unit (NICU); 2: To evaluate the prevalence of Extended spectrum  $\beta$ -lactamase (ESBL) production in these isolates.

**Materials and Methods:** All the blood samples of clinically diagnosed neonatal septicemia, received at the Department of Microbiology, at a tertiary care center, South India, for a period of one year were included for the study. *Klebsiella* species were isolated by following conventional standard microbiological technique. Antibiotic susceptibility testing was done by Kirby Bauer disk diffusion method. For the resistant isolates, extended spectrum  $\beta$ -lactamase detection was done by phenotypic confirmatory disk diffusion test (PCDDT) as per CLSI guidelines 2010.

**Results:** A total of 300 blood samples which met the study criteria were processed. Among these, Blood culture positivity rate was 68.33%, of which 100(49.5%) were *Klebsiella* isolates. Of these, 96 (96%) were *K.pneumoniae* and 4 (4%) were *K.oxytoca*. And out of these 100 *Klebsiella* isolates, 39 (39%) were ESBL producers, where 38 (97.43%) were *K. pneumoniae* and 1 (2.56%) was *K.oxytoca*.

**Conclusion:** The present study showed that *Klebsiella* species are the leading cause of neonatal septicemia. Prevalence of antibiotic resistant strains among *Klebsiella* species is predominant and deserves more consideration. Routine testing for ESBL production will guide for proper and judicious use of antibiotics. Implementation of appropriate infection control measures and adherence to them are crucial to control the spread of antibiotic resistant organisms.

**Keywords:** Neonatal septicemia, *Klebsiella* species, Extended spectrum  $\beta$ -lactamase (ESBL).

### Introduction

Neonatal sepsis is a clinical syndrome of infection characterized by signs and symptoms of systemic involvement during the first month of life.<sup>1</sup> It is further classified according to the time of onset of the disease as early onset (EOS: 0-3days of life) and late onset (LOS: after 72 hours of birth till one month).<sup>2</sup> In the developing countries like India, neonatal sepsis is a major cause for admission to the neonatal intensive care unit and it is a major cause of morbidity and mortality worldwide, accounting for about 5 million deaths annually.<sup>3,4</sup>

Isolation of microorganisms by blood culture is considered as a gold standard method for the diagnosis of neonatal septicemia.<sup>5</sup> If diagnosed early and treated aggressively, it is possible to reduce morbidity and mortality in cases of neonatal sepsis.<sup>6</sup> In most developing countries, Gram-negative bacteria are the major causative agent, and<sup>7</sup> *Klebsiella* is the predominant pathogen causing fulminant early onset neonatal sepsis.<sup>8-10</sup> The reported incidence of nosocomial neonatal sepsis in India ranges from 1.5-37%.<sup>11</sup> Although any pathogen may be acquired by the neonates in the hospital, *Klebsiella* has emerged as an important cause of neonatal nosocomial infection as well.<sup>11</sup>

Of particular concern is ESBL producing *Klebsiella* species vastly outnumbering the sensitive strains as the causative agent of neonatal septicemia. ESBL strains are those that possess plasmids which mediate resistance to

extended spectrum  $\beta$ -lactam drugs, due to the production of unique  $\beta$ -lactamase enzymes, referred to as extended spectrum  $\beta$ -lactamases or ESBL's thus narrowing the choice of antibiotics that can be used.<sup>9</sup>

### Materials and Methods

Blood samples received at our laboratory for a period of one year, between January 2010 and December 2010 from clinically suspected cases of neonatal septicemia were included for the study. History emphasizing on risk factors was taken from the patient's attendant. All these blood samples were processed according to standard protocol. They were considered negative if there was no growth after 7 days of aerobic incubation. Identification of *Klebsiella* species was done based on colony characteristics and standard biochemical reactions. Only samples which isolated *Klebsiella* species were further considered for the study. The isolates were speciated into *Klebsiella pneumoniae* and *Klebsiella oxytoca* based on the ability to produce indole.

Antibiotic susceptibility testing was done by Kirby Bauer disk diffusion technique using antibiotic disks procured from Himedia Laboratories Pvt. Ltd. Mumbai. Antibiotics tested were Ampicillin (10 $\mu$ g), Amoxicillin-Clavulanic acid (20/10  $\mu$ g), Cefuroxime (30  $\mu$ g), Ceftazidime (30  $\mu$ g), Cefotaxime (30  $\mu$ g), Ceftriaxone (30  $\mu$ g), Gentamicin (10  $\mu$ g), Amikacin (30  $\mu$ g), Ciprofloxacin

(5 µg), Levofloxacin (5 µg), Trimethoprim-sulfamethoxazole (1.25/23.75 µg), Imipenem (10 µg), Meropenem (10 µg) and Aztreonam (30 µg). The zone of inhibition was measured and interpreted according to CLSI 2010 criteria.

### Detection of ESBL Production

Extended spectrum β-lactamase detection in *Klebsiella* isolates was done by phenotypic confirmatory disk diffusion test, using disks Ceftazidime (30µg) alone and in combination with Clavulanic acid (30/10µg). The procedure was carried out as per CLSI guidelines.

A measurement of ≥ 5 mm increase in zone diameter for Ceftazidime with Clavulanic acid versus ceftazidime zone diameter when tested alone, confirms ESBL production. (CLSI, 2010)

### Results and Analysis

A total of 300 blood samples were collected from clinically suspected cases of neonatal septicemia. Out of 300 samples, 205 yielded the growth, with overall blood culture positivity rate of 68.33%.

Of these, 100 isolates were *Klebsiella* accounting for 49.5%, which were taken up for the study. Among them, 96(96%) were *K.pneumoniae* and 4(4%) were *K.oxytoca*.

**Table 1:** Age wise distribution of *Klebsiella* species

Age (days)	Species – No (%)		Total (%)
	<i>K.pneumoniae</i>	<i>K.oxytoca</i>	
0-3(EOS)	63(63)	03(3)	66(66)
4-28(LOS)	33(33)	01(1)	34(34)
Total (%)	96(96)	04(4)	100

EOS-Early onset septicemia, LOS-Late onset septicemia. *K.pneumoniae*-*Klebsiella pneumoniae*, *K.oxytoca*-*Klebsiella oxytoca*.

Out of 100 *Klebsiella* isolates, maximum number of cases were in the EOS group (0–3 days) i.e. 66% and 34% in the LOS group (4-28 days), with EOS to LOS ratio of 1.94:1. Among 66 isolates causing EOS, 63(63%) were caused by *K.pneumoniae* and 3(3%) by *K.oxytoca*. Among 34 isolates causing LOS, 33(33%) were caused by *K.pneumoniae* and 1(1%) by *K.oxytoca*.

In the present study 63% were males and 37% were females, with male to female ratio of 1.7:1.

EOS-Early onset septicemia, LOS-Late onset septicemia, PROM-Prolonged Rupture of Membranes, RDS-Respiratory Distress Syndrome, MAS-Meconium Aspiration Syndrome.

**Table 2:** Risk factors associated with Neonatal septicemia

Age (days)	Risk Factors – No. (%)							
	Preterm	RDS	PROM	Neonatal Hypoxia	MAS	Birth asphyxia	Prolonged labor	Refusal of feeds
0-3(EOS)	33(91.6)	22(95.65)	17(85)	16(100)	12(80)	12(92.3)	8(72.72)	2(22.22)
4-28(LOS)	3(8.33)	1(4.34)	3(15)	0(0)	3(20)	1(7.69)	3(27.27)	7(77.77)
Total	36	23	20	16	15	13	11	9

Several risk factors like preterm birth, respiratory distress syndrome, prolonged rupture of membrane, meconium aspiration syndrome and birth asphyxia were mainly associated with EOS than LOS.

In the present study, all *Klebsiella* isolates showed resistance to ampicillin which is indeed intrinsic resistance showed by the organism. Highest sensitivity was seen with Imipenem followed by Meropenem.

Among 100 isolates of *Klebsiella*, 39 were ESBL producers and 61 were non-ESBL producers. Of 39 ESBL producers, 38(97.43%) were *K.pneumoniae* and 1(2.56%) were *K.oxytoca*.

Out of 38 ESBL producing *Klebsiella pneumoniae*, 27 were isolated from EOS cases and 11 from LOS cases. The one ESBL producing *K.oxytoca* isolated was from LOS case.

### Discussion

Neonatal sepsis is a major cause for admission to the neonatal intensive care unit and *Klebsiella pneumoniae* is a major causative agent till date.<sup>4,12</sup> In the present study also, *K.pneumoniae* has been found to be the predominant pathogen accounting for 96% of the total isolates and is in line with other studies like Shubha et al., and Prabha Lal et al who reported 84.5%<sup>13</sup> and 97.1%<sup>14</sup> respectively. The ability of *Klebsiella pneumoniae* to spread rapidly amongst patients often leads to nosocomial outbreaks of infection especially in neonatal units.<sup>15</sup>

The incidence of ESBL producing strains among the clinical *Klebsiella* isolates has been steadily on the rise over the past several years and in India, high prevalence of ESBL producing *Klebsiella* strains has been reported by various groups, with a range of 6-87%, may be due to selective pressure imposed by extensive use of antimicrobials. Intensive care unit where the antibiotic use is heaviest is an important factor.<sup>12</sup> In the present study 39% of the isolates were ESBL producers which is comparable with Amar Ali et al<sup>16</sup> and Luzzaro et al<sup>17</sup> who reported 37.5% and 37.1% respectively. And majority of ESBL producers in the present study were in the EOS group (69%) suggesting that, babies in this age group are more susceptible.

ESBL production is frequently accompanied by multidrug resistance and hence its management has remained as a nightmare for neonatologists and microbiologists.<sup>18,19</sup> However, so far, ESBL producing *Klebsiella* strains are susceptible to carbapenems like Imipenem and Meropenem, which are the treatment of choice for ESBL producers.<sup>18</sup> This is echoed even in our study where highest sensitivity (84%) was recorded with Imipenem followed by Meropenem(79%).

**Table 3:** Antibiotic susceptibility pattern of the *Klebsiella* isolates

Antibiotics	Sensitive (%) n=100	Resistant (%) n=100
Ampicillin	00(00)	100(100)
Amoxicillin/Clavulanic acid	8(8)	92(92)
Cefuroxime	11(11)	89(89)
Cefotaxime	15(15)	85(85)
Ceftriaxone	19(19)	81(81)
Ceftazidime	23(23)	77(77)
Gentamicin	12(12)	88(88)
Amikacin	54(54)	46(46)
Ciprofloxacin	56(56)	44(44)
Levofloxacin	43(43)	57(57)
Trimethoprim-sulfamethoxazole	20(20)	80(80)
Imipenem	84(84)	16(16)
Meropenem	79(79)	21(21)
Aztreonam	46(46)	54(54)

**Table 4:** ESBL producers among *Klebsiella* isolates

<i>Klebsiella</i> species	Number of ESBL Producers (%) n=100
<i>Klebsiella pneumoniae</i>	38(38)
<i>Klebsiella oxytoca</i>	01(01)
Total	39(39)

ESBL- Extended Spectrum  $\beta$ -Lactamases, EOS-Early onset septicemia, LOS-Late onset septicemia.

**Table 5:** Age wise distribution of ESBL producing *Klebsiella* isolates

Age group	ESBL Producing <i>Klebsiella pneumoniae</i> (%) n=39	ESBL Producing <i>Klebsiella oxytoca</i> (%) n=39	Total (%) n=39
0-3 days (EOS)	27(69)	00(00)	27(69)
4-28 days (LOS)	11(28)	01(3)	12(31)

The infection control implications of ESBL producing *Klebsiella* species are under-recognized, so routine detection of ESBL producing microorganisms is required by reliable laboratory methods and therapeutic strategies to control infections in NICUs has to be carefully formulated.

### Conclusion

*Klebsiella pneumoniae* has emerged as a significant nosocomial pathogen in the neonatal units. With the recent increase in ESBL producing strains in the hospitals across the world, there is need to know the prevalence of ESBL producing *Klebsiella* species in cases of neonatal septicemia so as to formulate guidelines for appropriate antibiotic usage

and measures to control the spread of ESBL producers in neonatal units.

**Conflict of Interest:** None.

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**How to cite this article:** Anitha TK, Anuradha K. Neonatal sepsis due to ESBL producing *Klebsiella*. *Indian J Microbiol Res* 2019;6(2):113-6.