

Prevalence of Extended Spectrum of B-Lactamase Producing Gram Negative Bacteria in Septicemia Neonates in Tertiary Care Hospital

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Abstract: Emergence of extended spectrum β lactamases (ESBLs) producing strains of gram negative bacteria, as one of the leading cause of septicemia often complicates the clinical and therapeutic outcome. The present study was undertaken to investigate the prevalence of ESBLs in bacteria isolated from neonatal septicemia cases along with their antimicrobial sensitivity pattern. Blood samples were collected from 100 suspected cases of neonatal septicemia. Apart from susceptibility testing, all the gram negative isolates were subjected to phenotypic tests for ESBL production. Amongst the positive test samples (n = 40), 25 were gram negative rods. ESBL was detected in 6 isolates. Results indicate that routine ESBL detection should be made imperative and empirical use of third generation cephalosporin must be discouraged.

Keywords: Extended spectrum β lactamase, Drug resistance, Neonatal septicemia.

I. Introduction

Septicemia is one of the leading causes of neonatal mortality along with perinatal hypoxia. ^[1] Although the common factors associated with these infections are low birth weight, length of time spent in hospital, invasive procedures, surgery and also colonization by bacteria from hospital environment, a significant proportion of these septicemia babies are those, who were born unattended outside the hospital in unhygienic environment. ^{[2],[3],[4]}

The most common organisms responsible for these infections are multidrug resistant gram negative bacilli particularly members of the family Enterobacteriaceae and non-fermenting gram negative rods. ^{[2],[3]} Several outbreaks of septicemia by gram negative isolates have been reported and phenomenon of isolation of ESBLs producing isolates is not uncommon and is associated with increased mortality. ^[5] Further, as report of blood culture isolation and susceptibility are usually available after 72 hours or more, any delay in the initiation of correct empirical therapy or improper choice of antimicrobials cannot be justified. It is a common practice to use ampicillin and an aminoglycoside or a third generation cephalosporin in neonatal septicemia cases. ^[1]

This study on neonatal septicemia comprised of 100 neonates who were clinically suspected as septicemia, from the pediatric NICU ward, Government General Hospital, Guntur was conducted in the department of Microbiology, Guntur Medical College, Guntur

This study was designed to investigate the prevalence of ESBL producing gram negative rods in neonatal septicemia and to observe antimicrobial susceptibility pattern of ESBL and non ESBL producing gram negative isolates, which would enable formulation of appropriate antimicrobial policy for such patients

Aims And Objectives

1. To identify the prevalence of ESBL producers among the GramNegative isolates of neonatal septicemia.
2. To evaluate the anti-microbial susceptibility pattern for planning Strategyfor management.

II. Materials And Methods

This study on neonatal septicemia comprised of 100 neonates who were clinically suspected as septicemia, from the pediatric NICU ward, Government General Hospital, Guntur was conducted in the department of Microbiology, Guntur Medical College, Guntur spread over for a period of one year

Inclusion criteria

Neonates who were clinically suspected of septicemia

- Age < 28 days > 22 weeks of gestation and full term babies.
- Presence of three (3) or more clinical symptoms like refusal of feeds, lethargy, irritability, hypothermia, respiratory distress, jaundice, vomiting, apnoea, abdominal distension, hyperthermia, pustular skin lesions, seizures, sclerema, cyanosis, conjunctival discharge, bulging of anterior fontanellae, diarrhea.

Exclusion criteria

- Extreme prematurity <22 weeks of gestation
- Gross congenital anomalies
- Undergone surgery

Specimen collection

Blood sample collection

Venipuncture site was cleansed thoroughly with alcohol followed by Povidone-iodine applied in concentric circles moving outward from the center and again by alcohol. The skin was allowed to dry. About 1 ml of venous blood was drawn aseptically. Collected blood sample was inoculated into blood culture bottle containing 5-10ml brain heart infusion broth for isolation of the bacteria..

Processing of samples :

1. Culture and sensitivity

Isolation of bacteria from blood

Brain heart infusion broth with inoculated blood was incubated aerobically at 37⁰c for 12-18 hours. Sub culture was done in Biosafety cabinet on 5% sheep blood agar, chocolate agar and MacConkey agar were incubated at 37⁰c for 24 hours, and then at 7 days in between these time points, sub culturing was done only if there was visible turbidity.

Identification of the isolates

The isolates from blood were identified by colony morphology, gram staining, motility and the biochemical reactions as per the standard procedures.

Antibiotic sensitivity testing: Antimicrobial susceptibility testing was performed by Kirby Bauer disk diffusion method on Muller Hinton agar according to clinical laboratory standard institute (CLSI) recommendations.

Inoculum preparation

Using a sterile wire loop, 3-4 well isolated colonies of similar appearance from primary culture plate were inoculated into 2-3 ml of normal saline and subsequently emulsified the turbidity was adjusted so as to correspond to the 0.5 McFarland standards.

Inoculation of test plates

After adjusting the turbidity of the inoculum suspension, a sterile cotton swab dipped into the suspension. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The surface of the Muller Hinton agar plate was streaked with the swab evenly over in three directions. Using sterile forceps the appropriate antimicrobial disks were placed over the inoculated plate and not closer than about 25mm from disc to disc. Then the plates were incubated at 37⁰c for 18-25 hrs.

Interpretation of zone sizes

After overnight incubation, the plates were examined to ensure the confluent growth. Using the measuring scale ruler held on the underside of the plate the diameter of each zone of inhibition were measured in mm. The sizes of zone of inhibition were interpreted by referring to the CLSI standards and the organism was reported as susceptible, intermediate, or resistant to the agents that have been tested.

For gram positive bacteria, the discs tested were penicillin (10 μ g), ampicillin (10 μ g), vancomycin (30 μ g), ciprofloxacin (5 μ g), ciprofloxacin (30 μ g), gentamycin (10 μ g), piperacillin (100 μ g), linezolid (15 μ g) and cefotaxime (30 μ g).

For gram negative bacteria, the discs used were ampicillin (10 μ g), amoxicillin/clavunate (20/10 μ g), ciprofloxacin (5 μ g), gentamycin (10 μ g), and ceftriaxone (30 μ g), ceftazidime (30 μ g) and imipenem (10 μ g) piperacillin. (100 μ g)

Detection of ESBL production in gram negative isolates

ESBL production in gram negative isolates was further confirmed by double disk potentiation test and double disk synergy test as per CLSI standards. The ESBL producers were screened by both conventional and by Vitek system.

Phenotypic confirmatory test (Double disk potentiation test)

Ceftazidime (30 μ g) disk and a ceftazidime plus clavulanic acid (ca μ g + Caz 10 μ g) disk were placed at a distance of 20mm apart on a lawn of culture of the suspected ESBL producing clinical isolates on MHA. The plates were incubated at 37⁰c overnight.

Interpretation

The test organism was considered to produce ESBL, if the zone size around the ceftazidime + clavulanic acid increased >5 mm in comparison to the ceftazidime disk alone. This increase occurred because the β lactamases produced by isolates were inactivated by clavulanic acid.

Double disk synergy test

This was the first detection method described by Jarlier et al in 1988. In this test, the organism is swabbed on to a Mueller Hinton agar (MHA) plate. A susceptibility disk containing amoxicillin-clavulanate is placed in the center of the plate, and disks containing one of the oxyiminobetalactam antibiotics are placed 30mm (center to center) from the amoxicillin-clavulanate disk. Enhancement of zone of inhibition of the oxyiminobetalactam caused by the synergy of the clavulanate in the amoxicillin-clavulanate disk is a positive test result. This test was the reliable method for the detection of ESBLs.

Gram negative isolates were tested for following antimicrobial antibiotic discs and the zone size interpretative chart as comparable with CLSI standards.

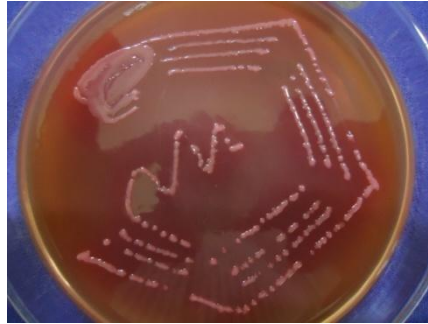
Antibiotics	Disc content μ g	Sensitive mm \geq	Intermediate mm	Resistant mm \leq
Gentamycin	10	15	13-14	12
Amikacin	30	27	15-16	14
Cefotaxime	30	23	15-22	14
Ceftazidime	30	18	15-17	14
Ceftriaxone	30	21	14-20	13
Ciprofloxacin	5	21	16-20	15
Imepenam	10	16	14-15	13
Piperacillin	100	21	18-20	17



Culture – Gram negative bacilli



Escherichia coli – LF colonies on Mac Conkey’s agar



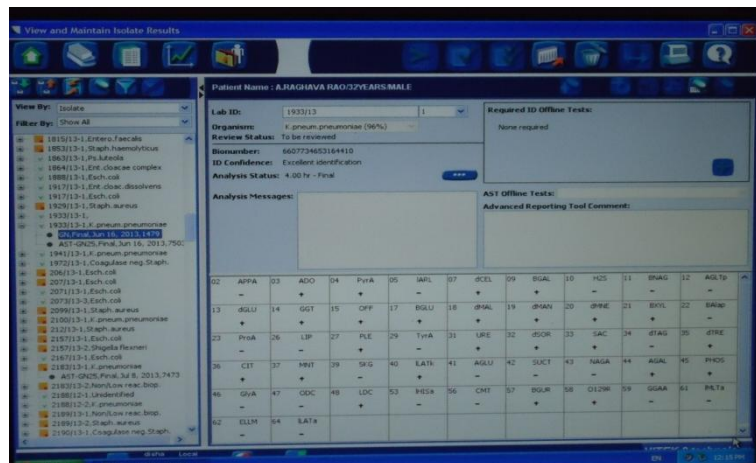
Klebsiella species – lactose fermenting colonies on MacConkey's Agar



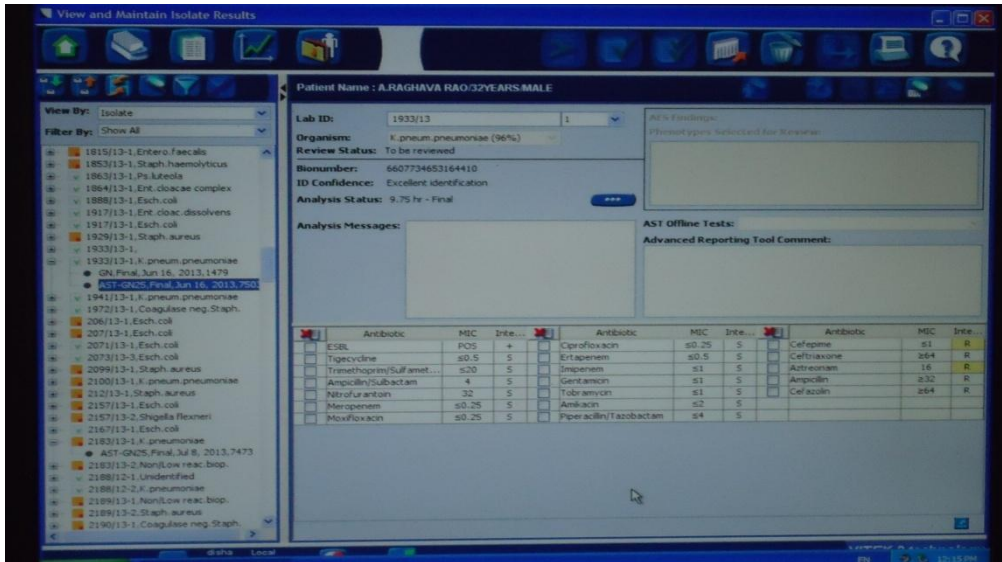
Antibiotic sensitivity test



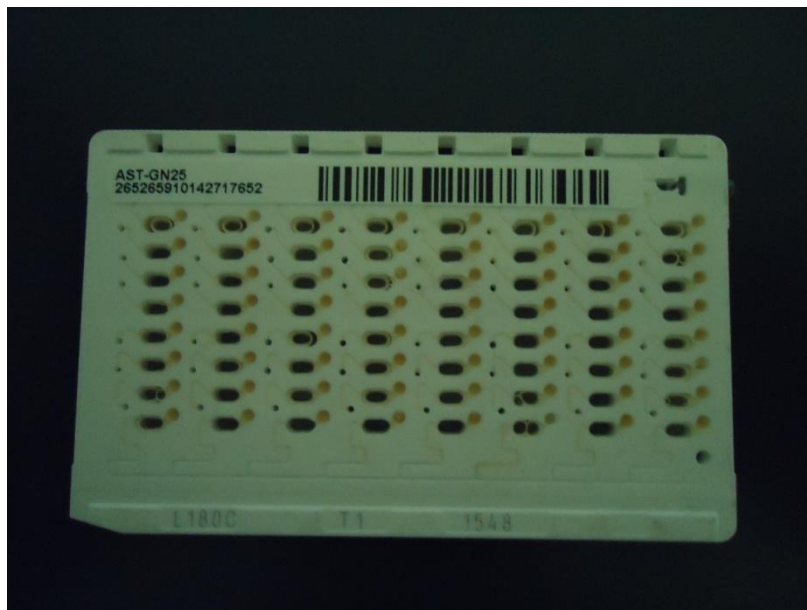
Vitek 2 –compact (Biomerix) system



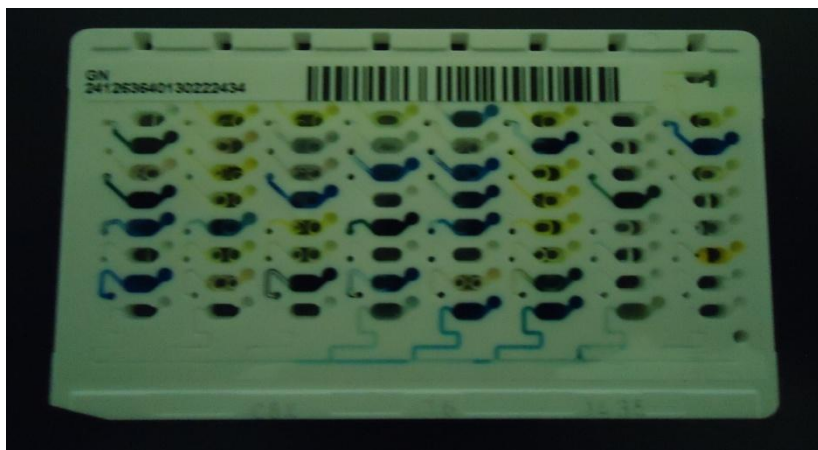
Biochemical reactions Klebsiellapneumonia



Culture sensitivity report of Klebsiellapneumonia with ESBL identification and minimum inhibitory concentration



Antibiotic sensitivity card



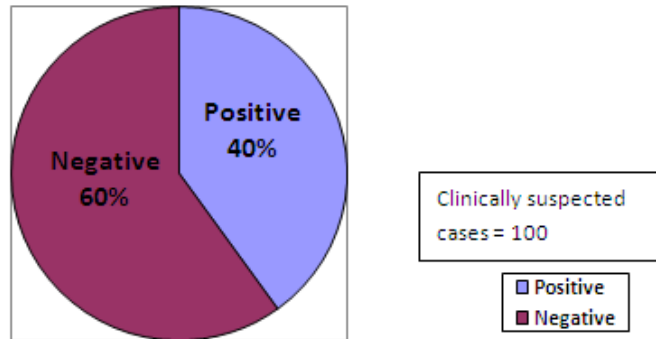
Microchip for identification of GNB

III. Results

Table 1 Out of 100 clinically suspected cases 40 were culture positive

Total cases	Culture positive	Culture negative
100	40	60
%	40%	60%

Blood culture positives



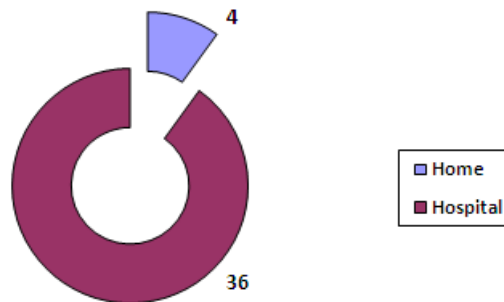
Place of Delivery

Out of 40 cases of neonatal septicemia 36 were hospital deliveries and 4 were home deliveries.

Table 4 Table showing place of delivery

Place of delivery	No. of neonates	%
Hospital	36	90%
Home	4	10%

Table showing place of delivery



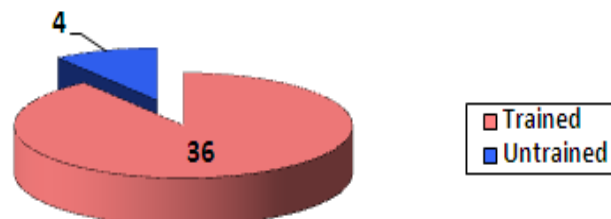
Persons conducting delivery

Among 40 cases of neonatal septicemia 36 (90%) babies were delivered by trained people like doctors, ANMs, Trained Dias, and 4(10%) babies by untrained Dais.

Table 5 Table showing persons conducting delivery

Persons	No. of neonates	%
Trained	36	90%
Untrained	4	10%

Persons conducted deliveries



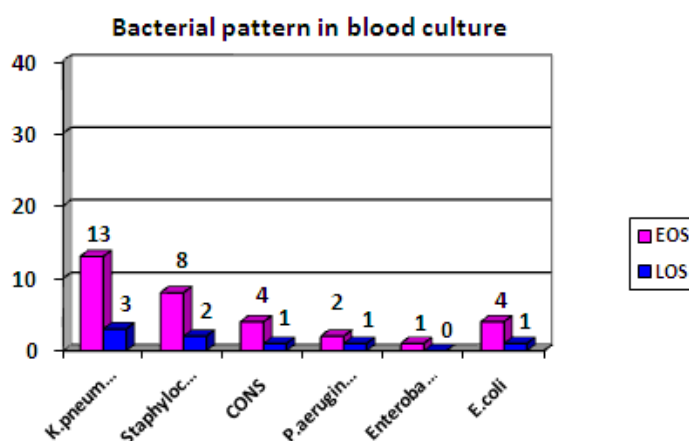
Distribution of organisms among culture positive cases

Among the 40 isolates, 25 were Gram negative bacilli (62.5%) and 15 were Gram positive cocci (37.5%). Out of the 25 Gram negative bacilli 16 were Klebsella (64%) 5 were E-coli (20%) 3 were Ps. aeruginosa (12%) and 1 was Enterobacter (4%).

Out of the 15 Gram positive cocci (37.5%) 10 were coagulase positive staphylococci (66.6%) and 5 were CONS. (Coagulase negative staphylococci)

Table 10 Percentage of blood culture positive isolates in neonatal sepsis.

Sno	Name of the bacteria	EOS	LOS	Total	Percentage
1	Klebsiella pneumonia	13	3	16	40%
2	Staphylococcus aureus	8	2	10	25%
3	CONS	4	1	5	12.5%
4	Pseudomonas aeruginosa	2	1	3	7.5%
5	Enterobacter	1	0	1	2.5%
6	Escherichia coli	4	1	5	12.5%



Antibiotic sensitivity pattern

All the Gram negative bacilli were sensitive to amikacin, gentamicin and piperacillin and Imipenem. Out of 25 GNB, 6 (24%) were ESBL producers. Out of 6 ESBLs Klebsella were 5 (83.3%) and E-coli was 1 (16.6%). Pseudomonas aeruginosa isolated in one case was found to be sensitive to piperacillin, gentamicin, amikacin and cefotaxime.

All the coagulase positive and negative staphylococci isolated were found to be sensitive to vancomycin, linezolid and piperacillin. Most of the coagulase positive staphylococci sensitive to penicillin, ceftriaxone, cefotaxime, gentamicin and amikacin.

Table 11 Antibiotic sensitivity pattern of organisms isolated from neonatal septicemia

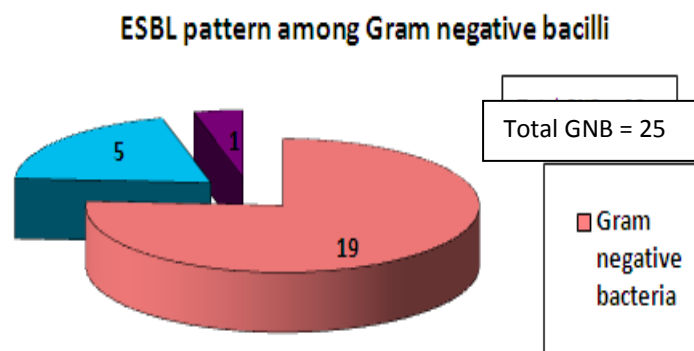
Antibiotics	Klebsiella 16		E. coli 5		Enterobacter 1		Pseudomonas 3		Staphylococcus 10		CONS 5	
	n	%	n	%	n	%	n	%	n	%	n	%
Gentamycin	14	87.5%	5	100%	1	100%	2	66.6%	10	100%	3	60%
Amicacin	16	100%	5	100%	1	100%	3	100%	10	100%	5	100%
Cefotaxime	11	68.75%	4	80%	1	100%	3	100%	7	70%	4	80%
Ceftriaxone	11	68.75%	4	80%	1	100%	3	100%	8	80%	4	80%
Ciprofloxacin	12	75%	4	80%	1	100%	2	66.6%	7	70%	2	40%
Imipenem	16	100%	5	100%	1	100%	3	100%	-	-	-	-
Pip / + Sulb	16	100%	5	100%	1	100%	3	100%	-	-	5	100%
Ceftazidime	10	62.5%	4	80%	1	100%	3	100%	8	80%	4	80%

Distribution of ESBLs among Gram negative bacilli.

Among 25 Gram negative bacilli, 6 were ESBLs (24%). Out of 6, Klebsiellapneumoniae 5 cases (83.3%) and E.coli 1 case (16.7%).

Table 12

Gram negative bacteria	ESBL	Klebsiella	E.coli
25 (62.5%)	6 (24%)	5 (83.3%)	1 (16.7%)



IV. Discussion

For the effective management of neonatal septicemia cases, study of the bacteriological profile with their antibiotic pattern plays a significant role.

In the present study, out of the 100 clinically suspected neonates, 40 (40%) were culture positive which correlated with Zakariya BP et al (41.6%) and RakheeAgarwal (42.7%) (**Table No. 1**)

Out of the 40 cases of neonatal sepsis 36 (90%) were hospital deliveries and 4 (10%) were home deliveries. The neonate with septicemia was significantly higher in those delivered at hospital. This could partly be explained by the type of organisms isolated, most of the isolates in this study was possibly hospital acquired infections. This study was higher than NeemaKayang et al, Tanzania 2010 where 70% were hospital deliveries and 30% home deliveries and also near to T.Sirisha, Tirupathi of 86% hospital deliveries and 14% home deliveries. (**Table No. 4**) Among the 40 cases of neonatal sepsis 36 babies (90%) were delivered by trained people like doctors, ANMs, trained dias and 4 babies (10%) were by untrained dias which was near to T.Sirisha, Tirupathi, 2012 where 78% babies delivered by trained persons and 22% by untrained. Klebsiellapneumoniae was found to be the predominant organism causing neonatal septicemia in this study. Klebsiellapneumoniae was isolated in 16 cases constituting 40% of the total blood culture isolates which correlated with B.Subitha, 40% and nearer to RakheeAgarwal, who isolated Klebsiella in 44.6% of cases. Chugh et.al has noticed that most of the publications have reported high incidence of Klebsiella septicemia of this organism emerging as the predominant causative organism, although this was not a universal phenomenon.

Monga et al had isolated Klebsiella in 41.2% of their cases followed by Staphylococcus aureus, E.coli and Pseudomonas aeruginosa. M.Singh et al in their study found Klebsiella pneumonia to be the commonest organism constituting 47.61% of the positive blood cultures.

Escherichia coli were isolated in 5 cases (12.5%) which correlated with RakheeAgarwal of 12.3%. Pseudomonas aeruginosa was isolated in 3 cases (7.5%) correlated with Mane AK et al, 2010 of Nagpur of 8.5%. Enterobacter cloacae were isolated in one case (2.5%) which was near to Waseem R et al, 2005 at Lahore.

Coagulase positive staphylococci were isolated in 10 cases (25%) of total blood culture isolates which was higher to Mane AK et al, 2010 of Nagpur of 14.2%. Coagulase negative staphylococci isolated in 5 cases (12.5%) correlates with Zakariya BP et al, of 12%.

NNPD also showed Klebsiellapneumoniae as the most frequently isolated pathogen (32.5%), followed by Staphylococcus aureus (13.6%) and Escherichia coli (10.6%).

In this study Klebsiella showed a high degree of sensitivity of (100%) to imipenem, piperacillin with tazobactam correlating with the study of RakheeAgarwal (2012) where Klebsiella shown 100% sensitivity to imipenem and 82.8% to Pip/Taz except 6 isolates, which were ESBL producers.

In this present study the sensitivity rate of Klebsiellapneumoniae to ceftazidime 62.5%, cefotaxime and ceftriaxone 68.7% , Escherichia coli showed 100% sensitivity to imipenem and piperacillin, Enterobacter cloacae showed 100% sensitivity to amikacin, cefotaxime, Ceftriaxone, ceftazidime, imipenem and piperacillin. Pseudomonas aeruginosa showed 100% sensitivity to cefotaxime, Ceftriaxone, imipenem and piperacillin which correlates with B.Subitha et al.

Coagulase positive staphylococci and CONS isolates were 100% sensitive to vancomycin, piperacillin, amikacin and linezolid which correlate with RakheeAgarwal, 2012, Hyderabad. (**Table No. 11**)

In the present study 6 (24%) out of 25 Gram Negative isolates were ESBL producers out of 6 ESBLs, out the 16 Klebsiella 5 (31.25%) were ESBL producers showed 68.75% sensitivity, and 1 E.coli showed 80% sensitivity to 3rd generation cephalosporins. The prevalence of ESBL among clinical isolates varies greatly worldwide and within geographic areas, and is rapidly changing over time. This increased prevalence of Enterobacteriaceae producing ESBLs creates a great need for laboratory testing methods that will accurately identify the presence of these enzymes in clinical isolates.[6] The DDST is the most widely used test due to its simplicity and ease of interpretation.[11] It is a reliable method for the detection of ESBLs. (**Table No12**)

V. Conclusion

The number of lives lost in the perinatal and neonatal period exceeds that of any other period in life of a similar duration. In order to sustain gains in child survival made in recent decades, attention must be focused on reduction of morbidity and mortality in the newborn period.

The evaluation of tests for neonatal sepsis is important because the infection may present a very serious threat to the baby. The mainstay for therapy for Neonatal Septicemia being appropriate supportive care, antibiotics used based on susceptibility testing of organism isolated. The high percentage of ESBL producing *Klebsiella* spp may be due to the selective pressure imposed by extensive use of antimicrobials in Intensive care unit, in which antibiotic use is heaviest and the potential for patient to patient transmission of organisms is greatest. Continuing surveillance of Neonatal infections, Local patterns and antibiotic sensitivity of pathogens is vital to determine trends in the infection improve reliability of the data and guide empiric antibiotic therapy & preventive measures. This study indicates that routine ESBL detection should be made imperative and empirical use of third generation cephalosporins must be discouraged.

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