Extended Spectrum β Lactamase Producing Lactose Fermenters Causing Neonatal Septicaemia in a Tertiary Care Center in Uttar Pradesh

Prem P Mishra[1], Dakshina Bisht[2], Ved Prakash[3], Anil Kumar[4], Varun Goyal[5]

ABSTRACT

Introduction: Extended-spectrum Beta-lactamase (ESBL) producing enterobacteriaceae are of escalating concern in today's antibiotic era especially in neonatal sepsis. This study was conducted to determine the prevalence of ESBL producing *E.coli* and *Klebsiella species* in neonatal sepsis.

Material and Methods: This cross sectional study included 382 samples with signs and symptoms of neonatal sepsis. Blood cultures were done and the isolates were identified using standard biochemical tests and antibiotic susceptibility testing was performed by Kirby Bauer method. Beta-lactamase productions of the isolates were tested by combined disc diffusion test.

Results: Out of 382 samples, 124 (32.46%) samples [males were n=78/227 (34.36%) and 46/155 (29.67%) in females] were culture positive. The culture positivity among the Early Onset Neonatal Sepsis and Late Onset Neonatal Sepsis cases were 47.38% (n=59/124) and 52.42% (n=65/124).; The most common risk associated factors were premature birth, low birth weight, caesarian section etc among EONS and Low birth weight, premature birth, invasive procedures etc among LONS. Among the etiological agents, *Staphylococcus aureus* (n=24/47) (51.06%) and Coagulase negative *Staphylococci* (CoNS) (n=14/47) (29.79%) were most frequently isolated among Gram positive and *Escherichia coli (*n=30/73) (41.09%) followed by *Klebsiella species* (n=23/73) (31.51%) among the Gram negative isolates. ESBL production was seen in n=24/53 (45.28%) isolates [*Escherichia coli* n=13 (54.17%) and *Klebsiella* species n=11 (45.83%)]. Colistin and Imipenem are the most sensitive antibiotics for *Escherichia coli* and *Klebsiella species*.

Conclusion: High prevalence of ESBL producing *E.coli* and *Klebsiella* species was recorded among neonatal septicaemic cases. Testing of ESBL should be regularly done to formulate the empirical therapy based on region.

Keywords: ESBL, Escherichia, Klebsiella, Neonates, Sepsis

INTRODUCTION

Neonatal septicaemia is the most frequent cause for admission of neonates to hospitals and has been implicated in high incidence of morbidity and mortality, 30 – 50% of neonatal deaths.[1] Around one million deaths a year occur in the neonatal period (0–28 days) are due to infection, accounting for over 25% of neonatal deaths worldwide out of which 99% occur in developing countries like India.[2] The increased mortality is generally considered not to be owing to augmented severity of the disease in neonates presenting with infections, but rather due to higher failure rates of treatment.

In developing countries like India, the multiple drug resistant (MDR) organisms causing neonatal septicaemia are increasing and in particular Extended Spectrum β Lactamase producing Enterobacteriacae are of utmost concern. ESBL producers are resistant to *β*-lactam antibiotics including third-generation cephalosporins and often exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones. AmpC β-lactamases are the cephalosporinases which are poorly inhibited by clavulanic acid. These are different from ESBLs by their capability to hydrolyze cephamycins. Thus, they pose a fearsome challenge for patient management with limited therapeutic options.

The common factors associated with these infections are low birth weight; prolonged hospitalization, invasive procedures, surgery and also colonization by bacteria from hospital environment, a significant proportion of these septicaemic babies are those, who were born unattended or ill attended in the hospital in unhygienic environment.

In developing countries *Escherichia coli, Klebsiella spp, Acinetobacter* are more accountable than Group B *streptococcus* and Coagulase negative *Staphylococci* (CoNS) in causing early onset neonatal septicaemia (EONS). *Klebsiella spp* and *Pseudomonas spp, Salmonella spp* precede Coagulase Negative *Staphlococcus (*CoNS) and *Staphylococcus aureus* in causation of Late Onset Neonatal Septicaemia (LONS). Numerous outbreaks of septicaemia by gram negative isolate especially the ESBL producers have been reported from different places out of which *Klebsiella* spp and *E.coli* are more prevalent than any other enterobacterial species.[3] Resistant bacteria are emerging worldwide as a threat to favorable outcome in the treatment of common infections in community and hospital settings.

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The resistance is plasmid mediated. These are transferable conjugative plasmids which often code resistant determinants to other antibiotics. The plasmid-mediated resistance against cephalosporins can spread among related and unrelated gram-negative bacteria. ESBLs are mostly the products of point mutations at the active site of TEM and SHV genes.[4]
A recent report from the Infectious Diseases Society of America listed ESBL-producing *Klebsiella* species and *Escherichia coli* as two of the six drug-resistant microbes to which new therapies are urgently needed.[5] Any delay in the instigation of accurate empirical therapy or improper choice of antimicrobials especially in neonatal sepsis can prove fatal. Nowadays it has become common practice to blindly use 3rd generation cephalosporins in neonatal septicaemic cases. Various studies has been conducted related to ESBL producers in urinary isolates but limited studies has been undertaken regarding ESBL producers in neonatal sepsis cases. Keeping in view the above facts, this study is undertaken to find the prevalence of ESBL producing *Klebsiella species* and *Escherichia coli* along with their antibiotic susceptibility pattern among the cases of neonatal sepsis, which will help in defining appropriate therapeutic options.

MATERIALAND METHODS

Sample size: A total of 382 samples were collected assuming 58% [6] ESBL producers among the isolates.

Study type and design: This cross sectional study was carried out in the Department of Microbiology of Rohilkhand Medical College and Hospital, Bareilly in association with Santosh Medical College Hospital, Ghaziabad from May 2015 to May 2017.

Inclusion criteria
Patients of age ≤ 28 days were included in the study
Greater than 30 weeks of gestation and full term babies with signs of septicaemia like lethargy, poor feeding, irritability, fever, vomiting, abdominal distension, jaundice, respiratory distress, hypothermia, cyanosis and convulsions.

Exclusion criteria
Patients other than neonates will not be included in the study.
Extreme prematurity less than 30 weeks of gestation.
Gross congenital anomalies.
Undergone surgery.

Parameters: The data regarding age, sex, risk factors, patient identification number were collected from the Medical Record Department.

Definitions: Early onset neonatal sepsis/septicaemia (EONS) which is defined as infection occurring in either the first 48-72 hours of life or the first week of life.
Late onset sepsis (LONS) is defined as sepsis occurring after 72 Hrs.

Sample Processing: Blood samples (1-2 ml) from the neonates were collected by venipuncture under aseptic precautions and the samples were inoculated on blood culture bottle containing Brain Heart Infusion (BHI) broth (Himedia, Mumbai). The broths were incubated aerobically at 37˚ C for 7 days/by Bactec method. The samples were subcultured onto 5% sheep blood agar and Mac Conkey agar.

Identification and sensitivity: The isolates on the agar plates were identified by colony characteristics, Gram staining, motility and standard biochemical tests. AST will be performed by Kirby Bauer disc diffusion method as per Clinical Laboratory and Standard Institute (CLSI)[7] using Mueller Hinton Agar plates (MHA) and commercially procured antibiotic discs (Himedia).

Screening and Confirmation of ESBLs: According to the CLSI guidelines by "disc diffusion method". The isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg), ≤ 25 mm with Ceftriaxone (30 µg), and ≤ 27 mm with Cefotaxime (30 µg) were identified as potential ESBL producers and confirmation were done by "combined disc diffusion method". This test was done by using a disk of Ceftazidime (30µg) alone and a disk of Ceftazidime + Clavulanic acid (30 µg/10 µg) is used. Both the disks were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. A difference in zone diameters with and without clavulanic acid of ≥ 5 mm confirmed ESBL production.[8]
Quality control was done by non-ESBL-producing organism (*Escherichia coli* ATCC 25922) and an ESBL-producing organism (*Klebsiella pneumoniae* ATCC 700603).

STATISTICAL ANALYSIS

The data were analyzed using SPSS software 17th version. Microsoft excel were used for totaling, percentage and frequency.

RESULTS

A total of 382 samples were processed during the study period out of which 124 (32.46%) samples were culture positive. Of the 124 cases of blood culture positive neonates the positivity in males were n=78/227 (34.36%) and 46/155 (29.67%) in females as shown in Figure 1.
The mean age of the neonates was found to be 1.95 days. Among the 382 suspected cases of neonatal sepsis, early onset neonatal sepsis (EONS) was seen among 205 (53.66%)

Sl. No		Culture positive cases	Non culture positive cases	Total
1	EONS#	59 (28.78%)	146 (71.22%)	205 (53.66%)
2	LONS##	65 (36.72%)	112 (63.28%)	177 (46.33%)
	Total	124 (32.46%)	258 (67.54%)	382 (100%)
# EONS = Early onset neonatal sepsis; ## LONS= late onset neonatal sepsis				

Table-1: Distribution of culture positive cases among EONS and LONS cases.

Sl.No	Etiological agents	Isolated from eons cases	Isolated from lons cases	Total	P value
Gram Positive Organism					
	Staphylococcus aureus	11	13	24	0.0004
	Coagulase Negative Staphylococci	01	13	14	
	Group B Streptococci	05	00	05	
	Enterococci	02	02	04	
Yeast like fungi		00	04	04	
Gram Negative Organism					
	Escherichia coli	22	08	30	0.028
	Klebsiella species	10	13	23	
	Pseudomanas aeruginosa	01	07	08	
	Proteus species	03	03	06	
	Citrobacter species	03	01	04	
	Acinetobacter	01	01	02	
	Total	59	65	124	

Table-2: Etiological agents isolated from different types of neonatal sepsis.

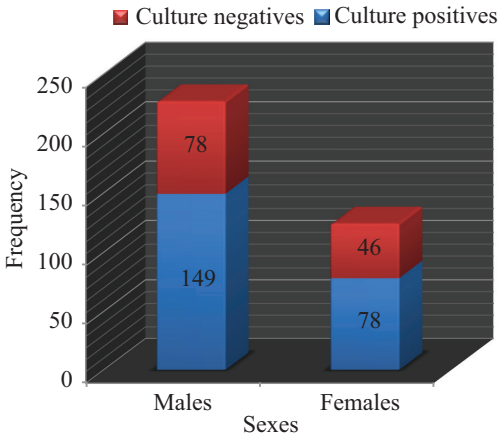


Figure-1: Distribution of males and Females in positive blood cultures

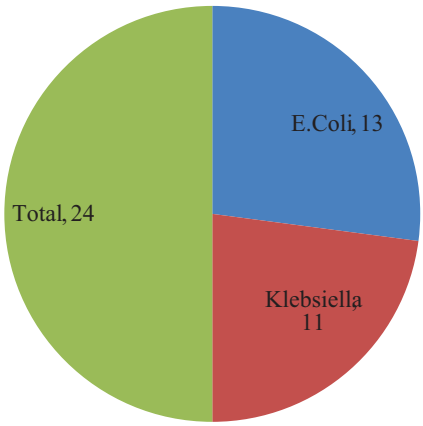


Figure-2: ESBL producing Escherichia coli and Klebsiella species.

patients while late onset neonatal sepses (LONS) were seen among 177 (46.33%) neonates. The culture positivity among the EONS and LONS cases were 47.38% (n=59/124) and 52.42% (n=65/124). The distribution is tabulated in Table 1. Amongst the cases of early-onset neonatal sepsis (EONS) the; The most common risk associated factors were Premature birth, low birth weight, caesarian section, Prolonged Rupture Of Membrane (≥ 12 hrs), followed by

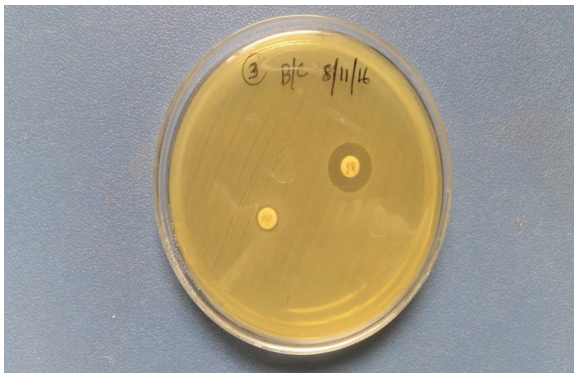
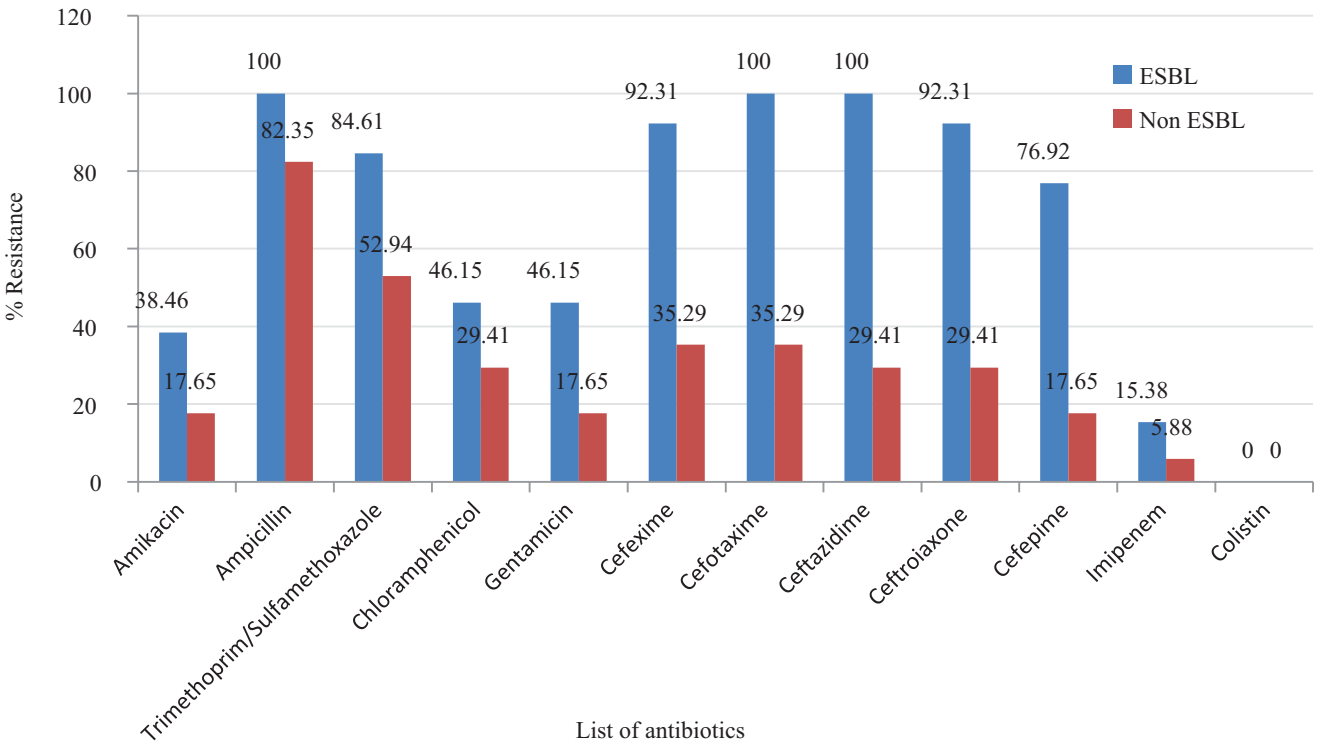


Figure-3: Combined disc diffusion test for detecting ESBL.

chorioamnionitis and epidural anesthesia. The risk factors associated with late onset neonatal sepsis (LONS) were Low birth weight, premature birth, invasive procedures and ventilator associated pneumonia. Many of the cases were found to have more than one of the risk factors.

The most common non specific clinical presentation that was recorded from the culture positive cases of neonatal sepsis that are included in this study were fever (71%), respiratory distress/asphyxia/pneumonia (51%), tachycardia (70%), Neonatal jaundice (16%) Lethargy and or poor cry (12%), followed by metabolic acidoisis and hypo or hyperglycemia. The specific signs included bulging fontanelle, tachypnea, GIT symptoms (like vomiting, diarrhea or abdominal distension), and less commonly hypotension, hepatomegaly and acute renal failure.

Gram negative isolates (n=73/124) comprised the majority (58.87%) of all culture positive neonatal sepsis cases, followed by Gram-positive (n=47/124) (37.9%) isolates and yeast like fungi (n=04/124) (3.22%). With regards to the etiological agents of neonatal sepsis, *Staphylococcus aureus* (n=24/47) (51.06%) and Coagulase negative *Staphylococci* (CoNS) (n=14/47) (29.79%) were found to be the most frequently isolated Gram positive organism from the specimen of neonatal sepsis cases. It was observed that proportion of CoNS was found more in LONS cases whereas Group B streptococci in EONS cases while *S. aureus* and



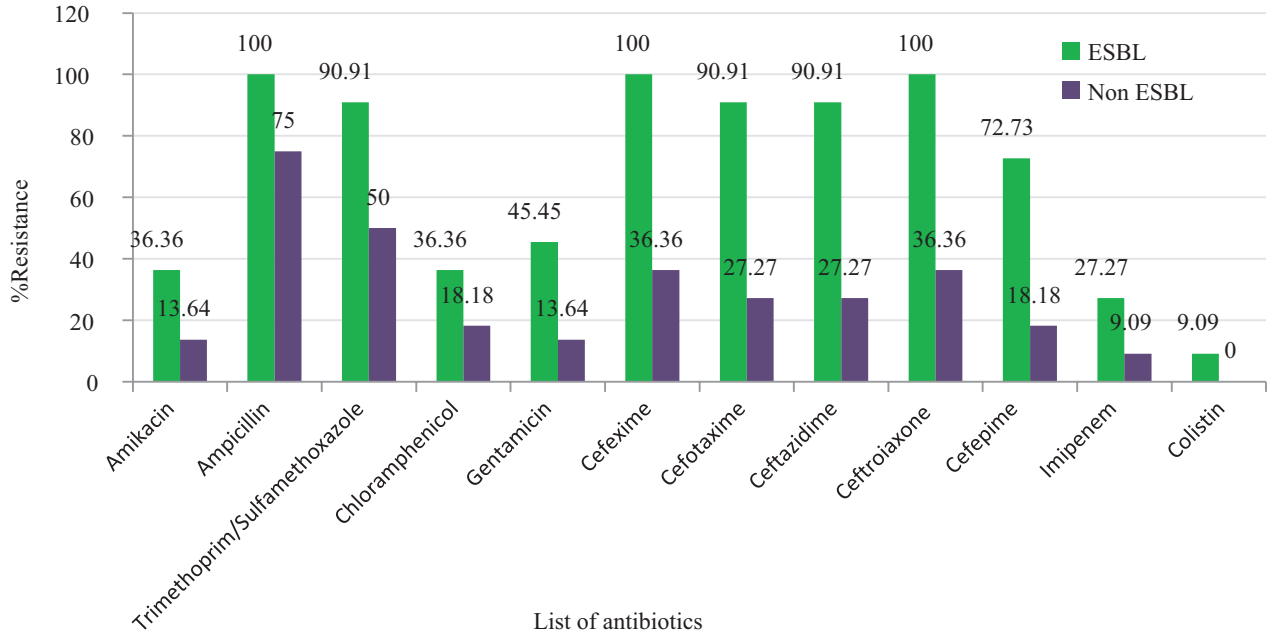


Figure-4: A and B: Resistance pattern among ESBL producing and Non ESBL producing isolates

Enterococci were isolated commonly in both types. This difference in distribution among various Gram Positive bacteria was found to be statistically significant (P<0.05).

Most common organism isolated were *Escherichia coli* (n=30/73) (41.09%) followed by *Klebsiella species* (n=23/73) (31.51%) among all the isolates from patients of neonatal sepsis. It was found that *E.coli* and *Citrobacter* were isolated more from EONS cases while *Pseudomonas* was more form LONS cases. *Klebsiella, Proteus* and *Acinetobacter* showed similar distribution among EONS and LONS cases. The difference in distribution was found to be statistically significant (P<0.05). The frequency of different etiological agents isolated from EONS and LONS is tabulated in Table 2. ESBL production was seen in n=24/53 (45.28%) of the 53 isolates of *Escherichia coli* and *Klebsiella species*. The ESBL production was seen more in *Escherichia coli* n=13 (54.17%) as compared to *Klebsiella* species n=11 (45.83%) as depicted in Figure 2. The ESBL detection test is displayed in Figure 3. The antibiotic susceptibility pattern of the ESBL producing and non ESBL producing *Escherichia coli* and *Klebsiella* species is displayed in 4 A and 4B.

DISCUSSION

Neonatal septicemia remains an important cause of

morbidity and mortality especially in developing countries along with the developed countries. The clinical diagnosis of the cases of neonatal sepsis is difficult as it presents with non-specific signs and symptoms and the risk factors are also innumerable. Keeping in mind about the evolution of the drug resistant strains, the accurate and timely identification of etiological agents and their resistance pattern are essential to combat the scourging effect of neonatal sepsis. According to the past studies, ESBL strains especially *Escherichia coli* and *Klebsiella species* have frequently been implicated in neonatal sepsis at tertiary care centers.[9, 10, 11]

The blood culture positivity rate among neonates in the current study was found to be 32.46%. A wide variation in the blood culture positivity has been reported over the years from different centers from our country. A higher isolation rate of 55.8% was reported by Yashwant Rao *et al* from a study conducted in Kanpur while a quite similar rate of 31.5% was observed by Ashish Khanna et al.[12,13] Of the 124 cases of blood culture positive neonates, the positivity in males were found to be 34.36% and 29.67% in females out of the total genders admitted. The male predominance is more than female because of higher proportion of males included in the study. In our study, it was found that late onset neonatal sepsis 52.42% was more common as compared to early onset neonatal sepsis which was found to be 47.58%. A similar data was reported in a study, which reported late onset neonatal sepsis (LONS) to be 51% as compared to early onset neonatal sepsis (EONS) of 49%.[14] The high incidence of Late onset neonatal sepsis is may be due to various predisposing factors like Low birth weight, premature birth, invasive procedures and ventilator associated pneumonia and in early onset neonatal sepsis was low birth weight, caesarian section, prolonged rupture of membrane (≥ 12 hrs) and chorioamnionitis as documented in our study. The health care system in our country as well as the socioeconomic status of the pregnant females in our country is poor. The most common non specific clinical presentation recorded in our study was fever, respiratory distress/asphyxia/pneumonia, tachycardia, neonatal jaundice and Lethargy and or poor cry.

Our study has shown a preponderance of Gram negative isolates (58.87%) among all culture positive neonatal sepsis cases as compared to Gram-positive isolates (37.9%) and yeast like fungi (3.22%). This is in line with the study conducted by Ashish et al in 2016 which reported 53.9% of cases by Gram Negative isolates. In contrast to our study, Thakur et al in 2016 reported 60% and 40% of the isolates to be Gram positive and Gram negative respectively.[13,14] With regards to the etiological agents of neonatal sepsis, *Staphylococcus aureus* (n=24/47) (51.06%) and Coagulase negative *Staphylococci* (CoNS) (n=14/47) (29.79%) were found to be the most frequently isolated Gram positive organism which is quite similar as compared to study conducted by *Kumar R et al* in Bihar.[15] The colonization of the skin and nasopharynx by CoNS and *S. aureus* in health care workers, the invasive procedures, lack of disinfection practice may lead to transmission of Gram positive organisms to neonates.

In the present study, *E. coli* species (n=30/73) (41.09%) and *Klebsiella* species (n=23/73) (31.51%) were most common Gram negative isolates. This is similar to the findings reported by Jyothi et al and those reported in the National Neonatal Perinatal Database.[16, 17] In contrast Khanna et al in their study found the *Klebsiella* species as predominant pathogen (20.2%) followed by Escherichia coli (14.6%).[13] We found *E.coli* (n=22/59) as the prime pathogen causing Early onset neonatal sepsis (EONS) and *Klebsiella* species (n=13/65) as the prime pathogen among Late onset neonatal sepsis among Gram negative isolates. The finding is similar to the study reported by Porta et al in 2017[18] which stated that *E. coli* as main cause of sepsis in the first 72 hours of life in term newborns. In our study significant number of ESBL production was seen among *Escherichia coli* (54.17%) and *Klebsiella species* n=24/53 (45.83%) which is inverse to the study conducted in Punjab which reported ESBL production in *Klebsiella* as 55.5% and *E.coli as* 46.2%.[13] This variation may be attributed to the different origin like community acquired or hospital acquired infections.

In our study 90-100% resistance against 3rd generation cephalosporins was observed among the ESBL producing strains of *Escherichia coli* and *Klebsiella species*. This outcome is in agreement with the study done by Islam et al which reported all ESBL positive strains of *E. coli* were resistant to cefotaxime, ceftazidime and ceftriaxone. We observed Colistin as the most sensitive drug followed by Imipenem (15-27% resistance).[19] The resistance among imipenem might be due to metallo betalactamase producers. The aminoglycosides like Amikacin and Gentamicin shown resistance of 36%-46% and resistance to Chloramphenicol was observed to be 36.36%- 46.15% for ESBL producing *E. coli* and *Klebsiella* species. The current study reported high resistance against Ampicillin (100%) and trimethoprim/sulfamethoxazole (84-90%) among the ESBL producing isolates. A Similar finding has been reported from Benin in 2015, they reported most effective antibiotics as imipenem (96.4% sensitive) and gentamicin (54.8% sensitive). High resistance was seen among ampicillin (94%) and trimethoprim/sulfamethoxazole (85.7%).[20]

CONCLUSION

There was high prevalence of extended spectrum beta lactamase producing *E.coli* and *Klebsiella species* among culture positive neonatal septicaemic cases in our study. Since the condition starts with fuzzy clinical signs, various signs and risk factors have been recorded. *E. coli* and *Klebsiella* species has been isolated more in cases of early onset neonatal sepsis as compared to late onset neonatal sepsis. It is recommended future studies to determine the risk factors for infection with ESBL producing pathogens. ESBL production is more seen in *E.coli* as compared to *Klebsiella* species. One of the limitations of this study was that it has not targeted the mortality rate associated with the ESBL producing organisms. It is recommended future studies should aim the multi drug resistance based on community acquired as well as hospital acquired infections among neonates. Testing for

ESBL is not done routinely in most of the health care set up in our country which leads to frightening spread of the domain of ESBL producing organisms in our country particularly in critical conditions. So the empirical therapy for neonatal sepsis should be regularly adjusted based on the prevalence of ESBL producing organisms in the affected area.

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