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Estimation Study of Septicaemic Patients in Neonatal ICU on Risk Factors and Microbiological Profile of Blood Samples of a Tertiary Care Hospital Dehradun in Uttarakhand

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Abstract – The objective of the present study is to Estimate risk factors and microbiological profile of blood samples of septicaemic patients. In the current study combined disc diffusion test (CDDT) recommended by CLSI was considered as the standard method. The validity of all other tests was compared with the standard tests.

Keywords – Septicaemic Patients, Neonatal, ICU, Microbiological Profile, Risk Factors

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INTRODUCTION

Septicemia is a main cause of childhood age mortality and morbidity. In neonatal septicemia, blood crops aren't always positive. The diagnostic of neonatal septicemia is supposed to be based on a mixture of physiological, hematological and other microbiologic data (colony morphology, gram staining, the biochemical characteristics and antibiotic sensitivity). Knowledge of infection pathogens in young children is important for the design of community level management strategies (1, 2).

In neonates, Septicemia and infants is a significant source of mortality and morbidity. Many risk factors both in neonates and infection prone children have been established which point to the need for childhood bacteriological control. As the major cause of septicemia in children were multi-drug-resistant, gram negative pathogens, great care is required in antibiotic therapy Selection (3-6).

Early starting neonatal sepsis (EONS) is characterized as a first 72-hour infection. Late onset neonatal sepsis is characterized by 72 hours and by 28 days as sepsis. EONS -and LONS are different types of propagation, etiology and care (7-9).

Moreover, the majority of peripheral health facilities in developed countries and pediatrists do not have facilities for bacterial cultivation. Early diagnoses and correct neonatal septicemia treatment could significantly reduce morbidity and mortality. The occurrence and frequency of septicemia in Sikkim neonates and infants are little understood. The current research was therefore conducted in Uttarakhand, in the neonatal ICU of a Dehradun tertiary hospital, to investigate risk factor evaluation and microbiological profile of blood samples of the patient septicaemic (10).

MATERIALS AND METHODS:

- **Study design:**

This Cross Sectional study was carried out in the Department of Microbiology of College & Hospital, Dehradun in Uttarakhand.

- **Sample size:**

Three eighty two samples were collected assuming 58% ESBL producers among the isolates.

$$n = \frac{4 \times p \times q}{L^2} = \frac{4 \times 58 \times 42}{(5.8)^2} = 290 \text{ (Minimum)}$$

n = Sample size

p = Prevalence rate of disease obtained from previous study i.e. 58% (11).

q = 100 – P = 100-58 = 42

L = Permissible error in the estimate of 'P' = 10% of 58 (absolute error)

Power of study = 100- Permissible error = 100-10 = 90

- **Criteria for selection of samples:**

Inclusion Criteria:

Septicaemic patients (Age ≤ 28 days.)

Greater than 30 weeks of gestation and full term babies with signs of septicaemia like lethargy, poor cry, irritability, fever/hypothermia, metabolic acidosis, gastrointestinal manifestations, jaundice, respiratory distress and other symptoms, tachycardia/bradycardia, tachypnea etc.

Exclusion Criteria:

- Patients other than neonates were not included in the study.
- Extreme prematurity less than 30 weeks of gestation.
- Gross congenital anomalies.
- **Data collection:**

The data regarding age, sex, risk factors, patient identification number were collected from the Medical Record Department. The parent/guardian of the patients voluntarily gave their consent for being the part of the study as it was a part of the routine diagnostic tests for the septicaemic neonates. They were explained the study protocol and a written consent were obtained from every parent/guardian before including them in the study.

- **Ethical aspects:**

This study was ethically approved by Institutional ethics committee of tertiary care hospital Dehradun in Uttarakhand. All procedures involving human subjects were carried out in compliance with the institutional research committee's ethical requirements as well as the 1964 Helsinki statement and its subsequent revisions. The tests were normal diagnostic procedures for a case of newborn sepsis.

- **Experimentation**

Blood Collection:

Blood samples (1-2 ml) from the patients with signs and symptoms of EONS (lethargy, temperature instability, poor cry, tachypnea, respiratory symptoms, Gastrointestinal manifestations, tachycardia/bradycardia, hypotension etc) or LONS (were temperature instability, apnea, tachycardia/bradycardia, dyspnea etc) were collected from the cases admitted in pediatric ward by following method:

Dorsal Hand Technique

The phlebotomist was asked to wash hands and place on gloves.

A 23g Butterfly Blood Collection Set attached to a 3-5 ml syringe is kept.

The hand with easily visible veins is selected and the site is warmed.

Transfer of Blood in Bottles:

The flip-off caps from blood culture bottles/BACTEC culture vials were removed. The blood is then transferred on to the blood culture bottle without switching the needle.

Subcultures:

The samples were subcultured onto 5% Mac Conkey agar, sheep blood agar, and Chocolate agar when blood cultures were done conventionally and monitored for 5 days when using Bactec method.

Isolation & Identification:

The isolates on the agar plates were identified by Colony characteristics, Gram staining, Motility, Standard biochemical tests and Antibiotic Sensitivity Testing (AST)

Phenotypic tests for the detection of esbls

► **Screening for ESBLs Production:**

The screening test for possible ESBL production according to 2015 CLSI guidelines considered that resistance to at least one of the prescribed antibiotics was positive (12).

- **Phenotypic confirmatory tests for ESBLs:**

► "Combined Disc Diffusion Method":

► MIC Reduction Test

- Determination of MIC by Agar Plate Dilution method
- Antimicrobial Agents Preparation
- Plates Preparation:

- Turbidity Standard for MIC Inoculum Preparation:
- Inoculation & Incubation of the Medium:
- Interpretation of results:
- By "Disc Antagonism Test (DAT)" for Inducible ß-lactamases (AmpC)

- **Double Disc Approximation Test for ESBL:**

Synergy was found between the Amoxicillin-Clavulanate (20 µg/10 µg) (Augmentin) disk and the 30-µg disk of the antibiotic Cephalosporin 3rd generation mounted on a Muller Hinton Agar (MHA) platform at a distance of 20 mm from center to center with the insulate test stated by Jarlier et al (298, 299). The simple extension of the cephalosporin inhibition area to the augmentin disk, which is described as positive for ESBL development.

- **Introduction & Principle:**

It comprised of a predefined quantitative gradient which is used to determine the MIC in mcg/ml of different antimicrobial agents against bacteria as tested on appropriate agar media, following overnight incubation. Nevertheless, the phenotypic confirmatory test does not identify all ESBLs. Some bacteria with ESBLs contain other β-lactamases that can mask ESBL production in the phenotypic test, resulting in a false negative test.

- **Genotypic detection:**

Multiplex Polymerase Chain Reaction (PCR) for detecting CTX-M, TEM and SHV genes:

► **Culture samples Selection:**

In 20 percent glycerol trypticase soy broth, the ESBL isolates were conserved and stored at -20°C. For genotypical studies these good Klebsiella spp and E. coli isolates were further processed. These isolates were sub-cultured with cefotaxime (2 µg/ml) Mueller Hinton agar and evaluated on their viability and cleanliness.

- **Statistical analysis:**

Statistical Package for Social Sciences was used to input and evaluate the data (SPSS, version 22). The categorical variables are presented as frequencies and percentages. Statistical analyses were performed using descriptive statistics such as frequency, percentage, mean and standard deviation. Chi square tests evaluated categorical results, while z proportion tests were used for the determination of the relationship between the classes. If the cell frequency was less than 5, Fischer's exact test was performed. A statistically important value of P =0.05 has been considered. For validity assessment of various phenotypic measures sensitivity, accuracy, positive and negative predictive values have been used are chi-square test, ANOVA test, T-test.

RESULTS:

- **Type of neonatal sepsis:**

The mean age of the neonates was found to be 1.95 days. The neonatal age of the patients ranged from 1 day to 28 days. Of the 382 neonatal sepses suspected in patients, Early Onset Neonatal Sepsis (EONS) was found among n=205 patients (53.66 percent), while N=177 (46.33 percent) of Late Onset Neonatal Sepsis (LONS) neonates were observed. Out of those there were 124 culture positive. The culture positivity was more in LONS cases 52.42% (n=65/124) as compared to EONS cases 47.38% (n=59/124). There is no significant association between the type of neonatal sepsis and blood culture positivity (p>0.05). The distribution of culture positivity in various types of neonatal sepsis is tabulated in Figure 1.

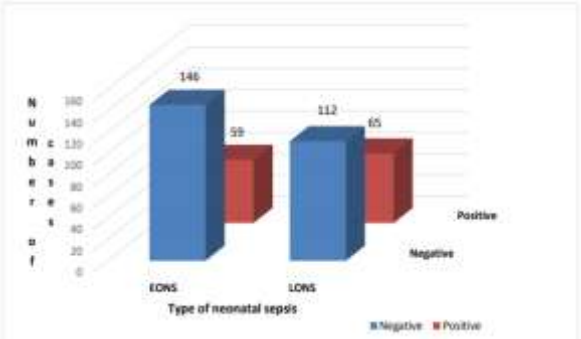

Figure 1: Culture Positive Cases among EONS and LONS cases

- **Risk factors:**

The most common risk factors associated with 177 cases of Late Onset Neonatal Sepsis (LONS) were invasive procedures (47.46%) (n=84/177) (p<0.01), prolonged antibiotic use (40.68%) (n=72/177) (p<0.01), low birth weight (23.16%) (n=41/177) (p<0.01), ventilator associated pneumonia & other respiratory conditions (22.03%) (n= 39/177) (p<0.01) while the less common factors were premature birth (19.21%) (n=34/177), caesarean section (14.69%) (26/177), chorioamnionitis (1.69%) (3/177) and pre-rupture of membrane (PROM) (0.56%) (1/177) (Figure 2)

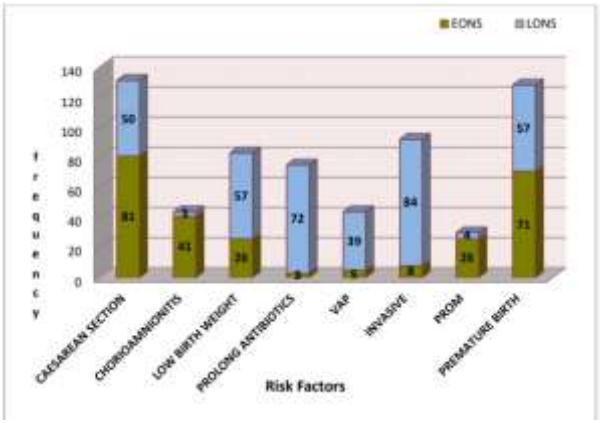

Figure 2: shows the risk factors associated with early and late onset neonatal sepsis

- **Signs and symptoms:**

The most common non-specific clinical presentation that was recorded from the clinically suspected cases of Neonatal Sepsis were fever or hypothermia (77.49%), tachycardia (68.8%), respiratory distress/asphyxia/pneumonia (31.41%), hypotension (26.70%), metabolic acidosis (20.94%), hypertension (10.21%), lethargy and or poor cry (9.69%), followed by neonatal jaundice (7.59%) and bradycardia (7.85%). The specific signs observed in the current study included bulging fontanelle (11.78%), GIT symptoms (like vomiting, diarrhea or abdominal distension) (9.95%), and less commonly hepatic and renal involvement (8.64%). The non-specific and specific signs and symptoms are tabulated in Figure 3.

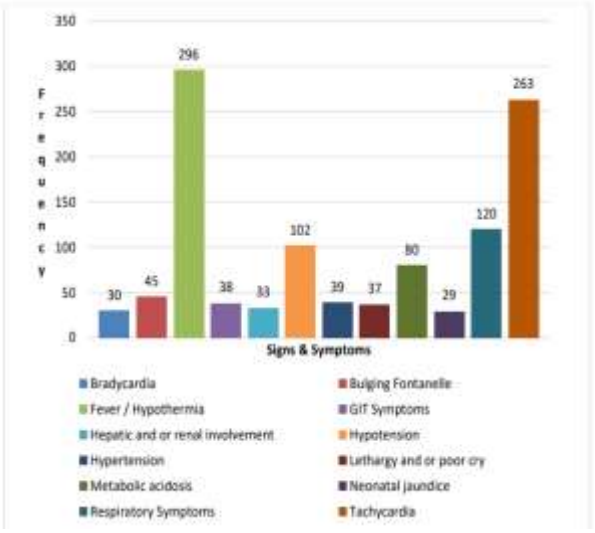

Figure 3: Signs and Symptoms Associated with Neonatal Sepsis

- **ETIOLOGICAL AGENTS:**

Among the Gram Positive isolates 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 organisms. The Gram Positive isolates from different types of sepsis are summarized in Figure 4.

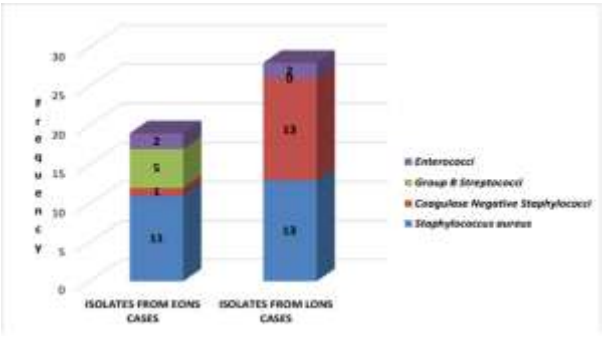

Figure 4: Gram Positive Bacteria Isolated From Neonatal Sepsis Cases.

Amongst the 73 Gram Negative isolates the most common organism isolated were E. coli (n=30/73) (41.09%) followed by Klebsiella spp (n=23/73) (31.51%) among all the isolates from patients of neonatal sepsis. A statistically significant differential ($p < 0.05$) differentiated distribution of Gram negative isolates with respect to neonatal septic types was found in Figure 5.

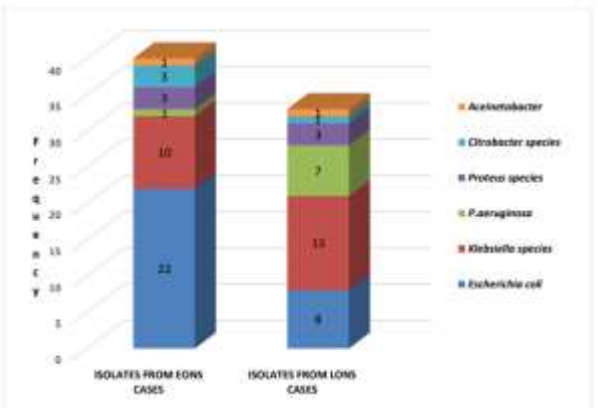

Figure 5: Gram Negative Bacteria isolated from Different Types of Neonatal Sepsis

Antimicrobial susceptibilities testing of escherichia coli (E. Coli) and klebsiella species isolates for screening of ESBL production:

We observed capricious susceptibilities among the Escherichia. coli and Klebsiella species against the antibiotics tested by Kirby Bauer method. [Figure 6, 7, 8]

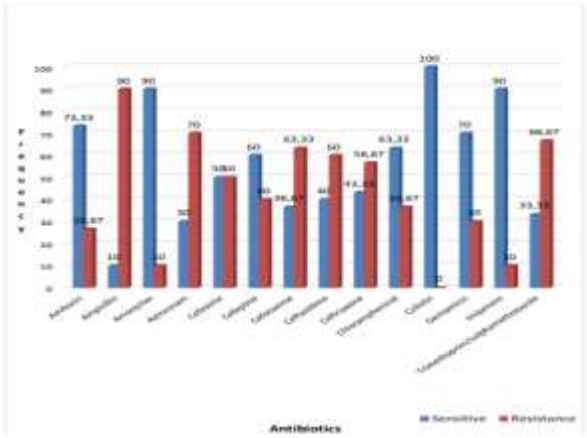

Figure 6: Antibiotic Sensitivity Pattern in Escherichia coli

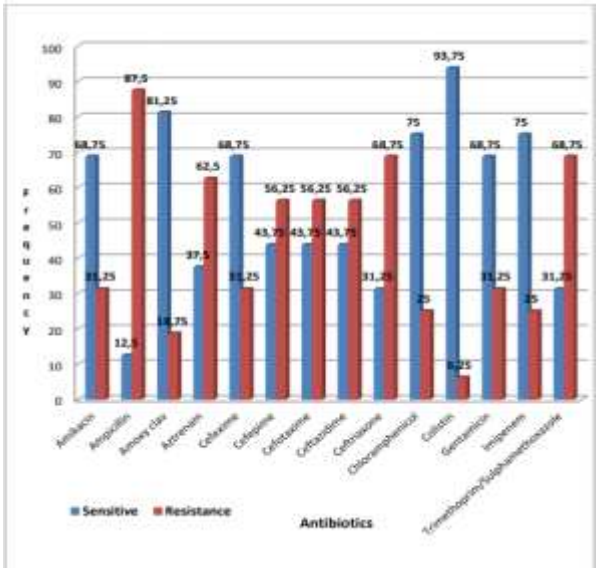

Figure 7: Antibiotic Sensitivity Pattern in Klebsiella pneumonia

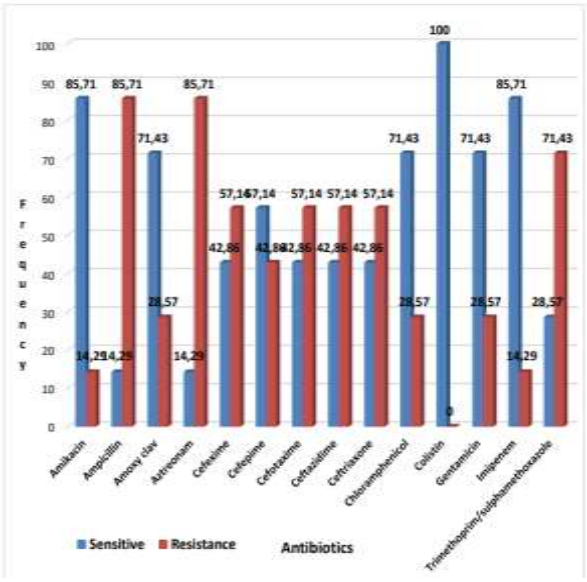

Figure 8: Antibiotic Sensitivity Pattern in Klebsiella oxytoca

N=29/53(54.72 percent) was shown to be antibiotic resistant to one or two of the four cephalosporin antibiotics during the screening test (ceftazidime, cefotaxime, ceftriaxone and aztreonam). N=27/29 (93.10.0%), cefotaxim in n=24/28 (82.76.0%), ceftazidim in n=24/29 (82.76.%) and ceftriaxone in n=25/29 (86.21.0%) isolates were observed for aztreonam resistance. This was also observed. Of the 30, n=16/30 E. coli isolates (53.33 percent) one or more of the screening agents were found resistant. N=12/16 (75%), of which all four screening agents were resistant. Of the 23 isolates of Klebsiella n=13/23 (56.52 percent) one or more of the screening agents were found to be resistant. Of those n=9/13 (69.23%), all four screening agents had resistance.

- **Confirmatory test:**

In the neonatal cases of sepsis with these bacteria, the prevalence of ESBL produced Escherichia coli and Klebesiella spp was 45.28 per cent. The prevalence was 43,33 percent and 47,83 percent for each of E. coli and Klebsiella spp. E.coli and Klebsiella spp isolation of ESBL producing EONS and LONS cases was found respectively to be 58.33% and 41.67%. ESBL detection among the isolates using the different tests is displayed in Figure 9.

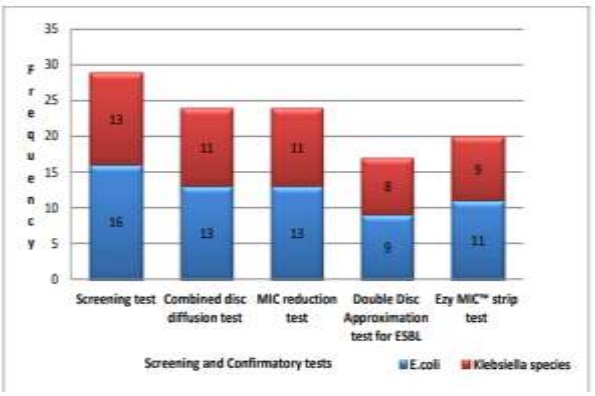

Figure 9: Screening and Confirmatory Tests among the Isolates

- **Antibiotic resistance pattern**:

All ESBL isolates generated by E. coli were tolerant Aztreonam (AZ) N=13/13(100%), Ceftazidime (CAZ) N=13/13 (100%), Cefotaxime (CTX) n=13/13 (100%) except one against Ceftriaxone (CTR) n=12/13 (92.31%) by disc diffusion test while non ESBL producing strains showed resistance pattern as Aztreonam (AZ) n=8/17(47.06%), Ceftazidime (CAZ) n=5/17 (29.41%), Cefotaxime (CTX) n=6/17 (35.29%) and Ceftriaxone (CTR) n=5/17 (29.41%). The statistically significant difference between the ESBL which produces E. coli and non-isolates (P <0.001) has been observed. ESBL producing *E.coli* showed higher resistance against Ampicillin

n=13/13 (100%) on the other hand non producers showed n=14/17 (82.35%) which is statistically insignificant (>0.05). The resistance pattern of *Escherichia coli* against the different antibiotics are displayed in Figure 10.

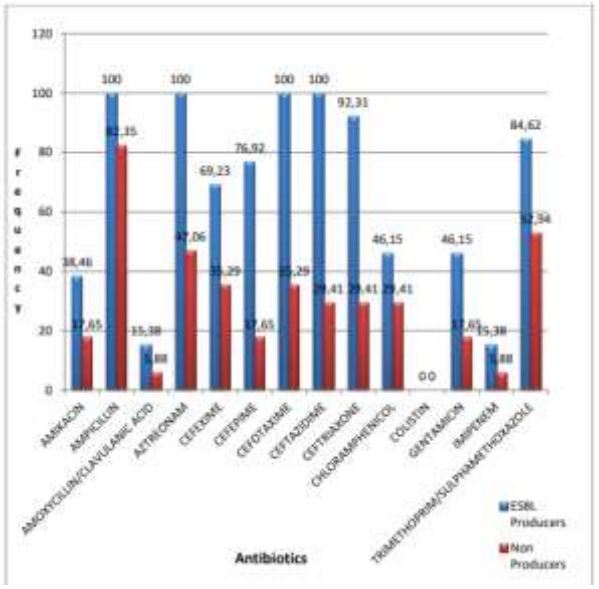

Figure 10: Resistance Pattern of ESBL Producing and Non-Producing Escherichia coli

- **Detection of *bla* genes by PCR**

In Klebsiella spp respectively, the prevalence in the genes of blaSHV, blaCTXM and blaTEM was found to be 69.23%, 61,54% and 38.46%. In 02 (15%) and in 5 (38%) and 3 (23.0%) isolates, two genes were found to coexist with three genes. Among these isolates, one gene could be detected bla single in 0,23%. In 1 (7.69 percent) of the Klebsiella isolates tested, the blaTEM and blaSHV gene were found singly. No blaCTX-M isolates contained in Klebsiella. There were also multiple occurrences in bla-EMT and bla-CTX-M in 1 (7.69 percent) of the genes and 4 (30.77 percent) of the isolates in Bla-SHV and in Blue-CTX-M. Coexistence among three (23,08 percent) isolates of blaTEM, bla SHV, bla CTX-M has been observed. Figure 11 displays the distribution and combination of blah genes in these isolates.

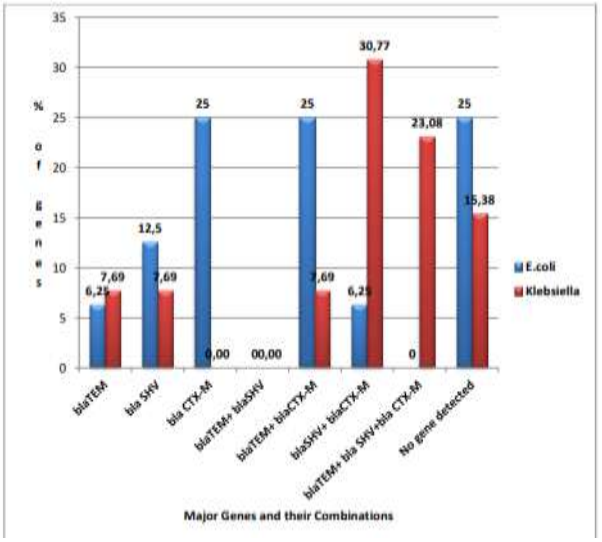

Figure 11: Distribution of Individual bla Genes and their Combinations

- **Prognosis among patients of neonatal sepsis:**

Out of the 24 neonatal sepsis cases with ESBL producing *E.coli* and *Klebsiella species,* the mortality was found in 4.17% (01 case) of cases which has been attributed to LONS case infected by ESBL producing *E.coli* as shown in Figure 12. The rest 85.83% has been discharged after recovery or in better condition to be taken to higher center.

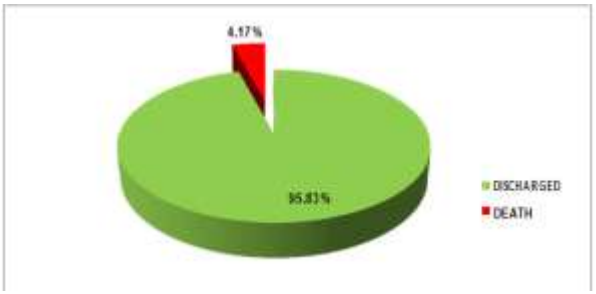

Figure 12: Prognosis of Neonates with Sepsis

CONCLUSION:

The n=2 isolation (6.89 per cent) were found to be coproduced for both ESBL and AmpC beta-Lactamase from 29 isolate using "disc antagonisms tests" for inducible AmpC ß-lactamases and Ezezy MICTM strips (EM078 and EM081)(himedia, Mumbai). ESBL isolated (n=1/16) (6.25%) and AmpC β-lactamases (7.69%) are produced from ENS, as are the ENS case isolates (n=1/16) and one Klebsiella spp (7.69%). Using these two methods, only the AmpC beta-lactamase producers, who were isolated from EONS cases, were identified as 01 (6.25%) E. coli (7.69%). The inducible AmpC frequency was 4/29 (13.79 percent). The E. Coli and Klebsiella proportions were statistically

negligible for AmpC only and ESBL+AmpC (p=1) by Z test.

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