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Study of Gram Negative Organisms in Neonatal Septicaemia and its Antibiotic Susceptibility Pattern

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Abstract: Introduction neonatal septicaemia describes any systemic bacterial infection in neonates documented by positive blood culture. Septicaemia remains a significant cause of morbidity and mortality in the newborn. So, determination of bacterial etiology and antibiotic susceptibility patterns of isolates from septicaemia in Neonatal intensive care units (NICU) is now crucial to abate neonatal mortality. This study was under taken to know the bacterial etiology of septicaemia in neonate and antibiotic susceptibility pattern of the isolates. Aims and Objective to isolate Gram negative organisms causing neonatal septicaemia and to know the antibiotic sensitivity pattern for various isolates. Materials and methods : the study was conducted from July 2013 to December 2013 in Microbiology Dept. of tertiary care teaching Hospital, India. Total 300 Blood culture samples were collected from clinically suspected patients of sepsis from Neonatal intensive care unit and processed by BACT/ALERT 3D automated blood culture instrument. Antibiotic susceptibility pattern of isolates was studied by Kirby Bauer Disc diffusion technique and resistance pattern identify. And Result is out of 300 suspected cases 93 (31%) were culture positive. Of the 93 isolated organisms, 42(45.1%) were Gram positive organisms. isolates of Candida species were 3(3.23%) and Gram negative organisms 48(51.61%) which includes - *Klebsiella pneumonia* 26(27.9%), *E.coli* 14(15.05%), *Acinetobacter baumannii* 2(2.15%), *Pseudomonas* aeruginosa 4(4.3%), *Proteus mirabilis* 2(2.15%). In their antimicrobial susceptibility pattern gram negative isolates are most sensitive to polymyxin, levofloxacin, Imipenem, Piperacillin –tazobactam (61.53%), Amikacin (21.51) Discussion and conclusion: neonatal septicaemia is an important cause of neonatal mortality in a developing country like India. Because of emerging drug resistance and use of rational/irrational multidrug regimens in treatment of various infections have led to development of multi drug resistant organisms. Thus it has become important to have updated knowledge of the current trend of organisms involved in pathogenicity and their patterns of drug resistance.

Key words: Septicaemia · Antibiotic Susceptibility · Drug Resistence · Neonates

INTRODUCTION

Neonatal septicaemia describes any systemic bacterial infection in neonates documented by positive blood culture. It is an important cause of morbidity and mortality among neonates generally [1].

Neonatal sepsis may be classified accordingly to the time of onset of the disease, as early-onset (EONS) and late-onset (LONS) neonatal sepsis [2]. Early onset sepsis (EOS) (Less than 72 hrs) infections are caused by organisms prevalent in the maternal genital tract or in the delivery area. The predisposing factors include low birth weight (LBW), prolonged rupture of membranes, foul smelling liquor, multiple per vaginal examinations, maternal fever, difficult or prolonged labour and aspiration of meconium. EOS manifests frequently as pneumonia and less commonly as or meningitis.

Late onset sepsis (LOS) (Greater than 72 hours) infections are caused by the organisms thriving in the external environments of the home or the hospital. The infection is often transmitted through the hands of the care providers. The presentation is that of pneumonia or meningitis. The predisposing factors include Low Birth Weight, lack of breastfeeding, poor cord care, superficial infections (Pyoderma, umbilical sepsis), aspiration of feeds and disruption of skin integrity with needle pricks and use of intravenous fluids [3].

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The causative microorganisms may vary not only from place to place but also from time to time in the same place. The Exact reason is not known but geographic, socioeconomic, seasonal and prevalent use of various antibiotics may play an important role. In developed countries group B Streptococci and Coagulase negative Staphylococci are the most common cause for early onset and late onset sepsis respectively. However in the developing countries, these organisms are rare with an entirely different bacterial spectrum. This bacteriological profile of neonatal septicaemia is constantly under change with advances in early diagnosis and treatment. Thus the rational protocol for sepsis management must be based on adequate knowledge of causative organism and their antibiotic sensitivity pattern in the related area [4]. Many studies have been conducted in India and other countries to find out the prevalence of septicaemia in neonatal age group. The present study undertaken to assess the role of Gram negative organism in septicaemia in neonates along with antimicrobial susceptibility pattern of isolated organisms.

MATERIALS AND METHODS

The study was conducted from July 2013 to December 2013 in tertiary care teaching Hospital, Ahmedabad. Total 300 Blood culture samples were collected from clinically suspected patients of sepsis from Neonatal intensive care unit

A detailed history was taken and Performa was filled for each Patient documenting age, sex , socioeconomic status, address, duration of illness along with artificial intervention, birth weight and any resuscitative procedures done. Venous blood was collected aseptically before initiation of antibiotic therapy [5].

Blood samples were collected with sterile syringe and needle after thorough cleaning of the venous site with 70% alcohol and subsequently followed by povidone iodine. And again with 70% alcohol then the rubber cap of the BacT/ALERT culture bottle were immediately cleaned with 70% alcohol. Then used needle was replaced with a new needle and then, the venous blood was injected into this bottle containing Brain heart Infusion broths in the ratio of one part of blood to five parts of the broth. The blood culture broths were immediately sent to the laboratory, where they were incubated at 37°C for 7 days [6].

The bottle loaded in Bact /ALERT Instruments which is automated systems that incubate, shake and monitor culture bottles for signs of microbial growth, where they were incubated at 37°C for 7 days.

Processing of Specimen for Bacterial Pathogen: If microbial growth appear then instrument give signal positive and if microbial growth not appear than after 7 days the sample declare negative if signal positive than unload bottle and make smear and gram staining and subculture on MacConkey, Blood and Chocolate agar media and incubated in appropriate temperature and atmospheres according to standard guidelines [6].

Second Day Follow Up: All inoculated plates were observed next day and the colony characteristics were identified by using the standard microbiological procedures like Gram staining , wet preparation for motility and Biochemical reactions as described in Practical Microbiology of Mackie MacCartney 14th volume [7]. After the isolation of bacteria, antibiotic sensitivity testing was done by Kirby-Bauer disc diffusion method on Muller Hinton (MH) agar as per CLSI recommendations [8].

And resistance pattern identify like ESBL by phenotypic combine disk method and Metallobetalactamase by phenotypic combine disk method. we also identify Amplified cephalosporinase by Modified Three-Dimensional Test (MTDT)

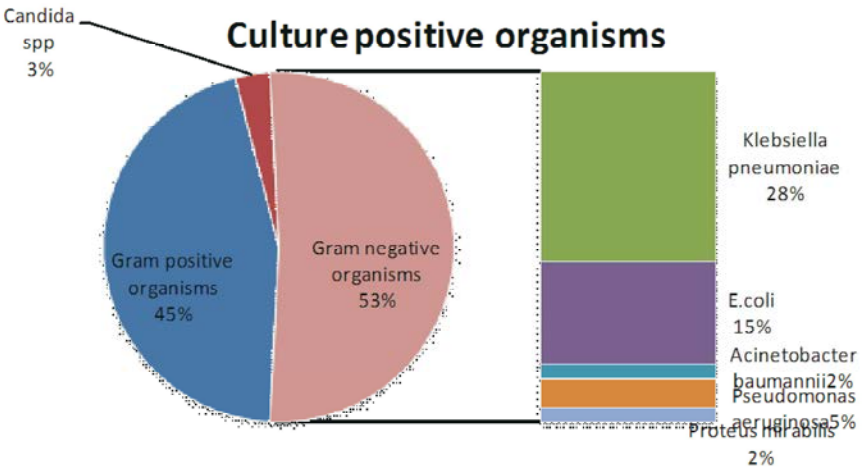
RESULTS

The study was conducted at Department of Microbiology in Tertiary care hospital Ahmedabad. The Blood culture specimen in BacT/Alert Bottles were received of suspected neonatal septicaemia cases from July 2013 to December 2013. Results and observations made from study are described below.

Blood Culture Positivity Rate: Out of 300 suspected cases 93 (31%) was culture positive.

Of the 93 isolated organisms, 42(45.1%) were Gram positive organisms that include - Coagulase negative *staphylococcus aureus [CONS]* 38(40.86%), *Staphylococcus aureus* 3(3.2%), *Enterococcus fecalis* 1(1.07%) ; isolates of *Candida* species were 3(3.23%) and Gram negative organisms 48(51.61%) which includes - *Klebsiella pneumoniae* 26(27.9%), *E.coli* 14(15.05%), *Acinetobacter baumannii* 2(2.15%), *Pseudomonas* aeruginosa 4(4.3%), *Proteus mirabilis* 2(2.15%).

Distribution Ofisolated Organisms



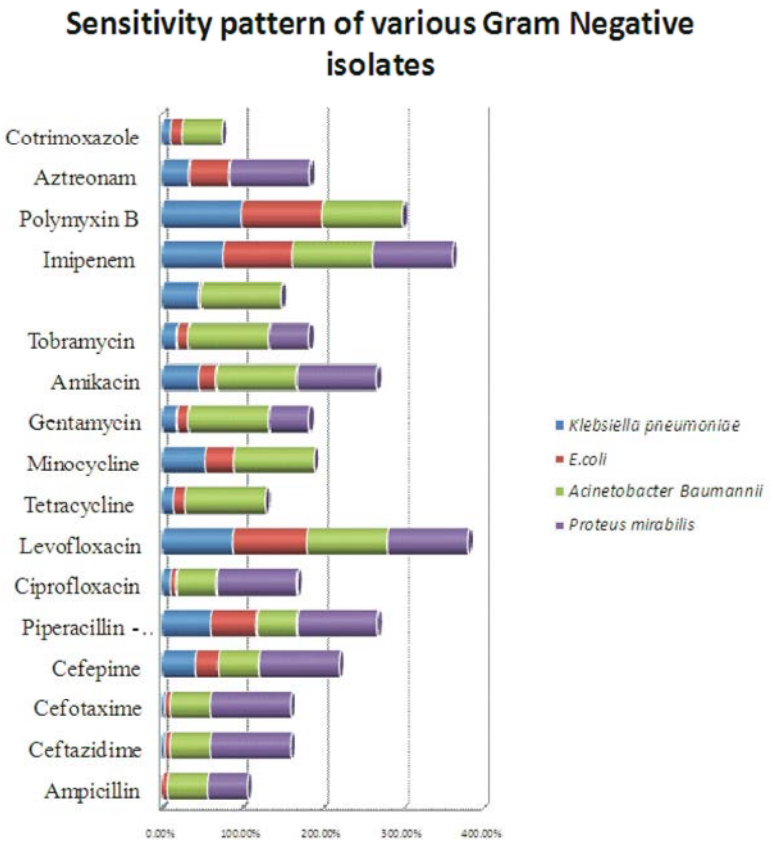
Antibiotic Susceptibilty Pattern: In antibiotic sensitivity test *Klebsiella pneumoniae* is highest sensitive to Polymyxin(100%) followed by Levofloxacin (88.4%) , Imipenem (76%), Piperacillin-tazobactam(61.53%) ,Amikacin (46.51%) and least sensitive to Cephalosporine and Cotrimoxazole.

In case *E. coli* is highest sensitive to Polymyxin(100%) followed by Levofloxacin (92.85%) , Imipenem (94%), Piperacillin-tazobactam(61.53%), Amikacin (21.51%) and least sensitive to Ampicillin, Cephalosporine and Cotrimoxazole.

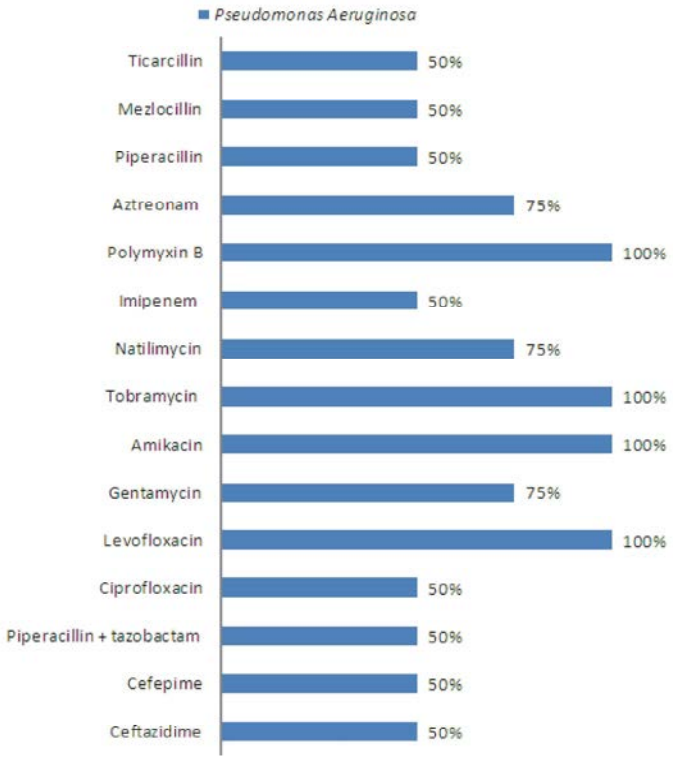
Pseudomonas aeruginosa 100% Sensitive to Polymyxin B and levofloxacin, Amikacin, 75% sensitive to Aztreonam, Netilimycin and 50% sensitive to Imipenem, piperacillin-tazobactum, Ciprofloxacine. and less sensitive to Mezlocillin and piperacillin and Ceftazidime.

Resistance Pattern: From all *Klebsiella pneumoniae* isolate 61.53% were ESBL producer, 19.23% Carbapenemase producer and 15% AmpC Beta Lactamase producer. In case of *E.coli* 61.53% ESBL producer, 14.28% Carbapenemase producer and 15% AmpC Beta Lactamase producer. In *Pseudomonas aeruginosa* isolates 50% MBL producer.

Antibiotic	*Klebsiella Pneumoniae*	*E.coli*	*Pseudomonas aeruginosa*	*Acinetobacter* spp.	*Proteus mirabilis*
Ampicillin	-	1(7.14%)	-	1(50%)	1(50%)
Ceftazidime	1 (3.84%)	1(7.14%)	2(50%)	1(50%)	2(100%)
Cefotaxime	1 (3.84%)	1(7.14%)	-	1(50%)	2(100%)
Cefepime	11 (42.30%)	4 (28.57%)	3(75%)	1(50%)	2(100%)
Piperacillin -tazobactam	16 (61.53%)	8 (57.14%)	2(50%)	1(50%)	2(100%)
Cefepime -tazobactum	-	-	2(50%)	-	-
Ciprofloxacin	3 (11.53%)	1(7.14%)	2(50%)	1(50%)	2(100%)
Levofloxacin	23(88.4%)	13 (92.85%)	4(100%)	2(100%)	2(100%)
Tetracycline	4(15.38%)	2 (14.28%)	-	2(100%)	-
Minocycline	14(53.8%)	5 (35.71%)	-	2(100%)	-
Gentamycin	5 (19.23%)	2 (14.28%)	3(75%)	2(100%)	2(50%)
Amikacin	12 (46.51%)	3 (21.42%)	4(100%)	2(100%)	2(100%)
Tobramycin	5 (19.23%)	2 (14.28%)	4(100%)	2(100%)	1(50%)
Natilimycin	-	-	3(75%)	-	-
Chloramphenicol	12 (46.15%)	6 (42.85%)	-	2(100%)	-
Imipenem	20 (76.92%)	12 (85.7%)	2(50%)	2(100%)	2(100%)
Polymyxin B	26(100%)	14(100%)	4(100%)	2(100%)	-
Aztreonam	9 (34.61%)	5(50%)	3(75%)	-	2(100%)
Cotrimoxazole	3 (11.53%)	2 (14.28%)	-	1(50%)	0
Piperacillin	-	-	2(50%)	-	-
Mezlocillin	-	-	2(50%)	-	-
Ticarcillin	-	-	2(50%)	-	-
Total	26	14	4	2	2



Sensitivity Pattern of *Pseudomonas aeruginosa*



DISCUSSION

For the effective management of neonatal septicaemia cases, study of the bacteriological profile with their antibiotic pattern plays a significant role. In this study, blood culture positivity rate in neonatal septicaemia cases was 31%, Similar study conducted in Ahmedabad in other institute by Rathod *et al*. [9] in 2011 had positivity rate of 17.09%. The results from other institute in different districts in Gujarat were similar. In Bhavnagar study conducted by Desai *et al.* [10] had culture positive rate of 46% in Surat study by Bhatt *et al*. [11] had rate of 56% and shah *et al.* [12] in surat had rate of 20%, in Amritsar study conducted by Poonam *et al*.[13] in 2011 had rate of 42.12% . Varied culture positivity rate may be due to the fact of use of antibiotics in some centres before collection of blood for culture and possibility of infection with anaerobes.

Out of 300 suspected cases 93 (31%) was culture positive.

Of the 93 isolated organisms, 42(45.1%) were Gram positive organisms that include - Coagulase negative *staphylococcus aureus* [*CONS*] 38(40.86%), *Staphylococcus aureus* 3(3.2%), *Enterococcus Fecalis* 1(1.07%); isolates of *Candida* species were 3(3.23%) and Gram negative organisms 48(51.61%) which includes - *Klebsiella pneumoniae* 26(27.9%), *E.coli* 14(15.05%), *Acinetobacter baumannii* 2(2.15%), *Pseudomonas* aeruginosa 4(4.3%), *Proteus mirabilis* 2(2.15%).

A comparison with meta analysis of data from 1980 to 2013 of middle and low income countries conducted by Donald *et al*. [14]. It shows globally as well as in south east asian region prevalence of Gram negative organisms is greater than gram positive. Our study had comparable results with prevalence of gram negative organism being 51.61%. Amongst gram negative *Klebsiella pneumonia* is more prevalent in south East Asian region while Global data shows prevalence of *E.coli* slightly more than Klebsiella. This study showed increased prevalence of *Klebsiella pneumoniae* (27.9%) which can be compared to south East Asian region data (45% prevalence of Klebsiella spp) [14]. Another study conducted in Gujarat in Bhavnagar from 2006 to 2008 and in Ahmedabad in 2011[15]shows similar results with preponderance of gram positive organisms. Klebsiella spp were the most common gram negative organisms isolated in all the studies conducted and also in our study.

Antibiotic Susceptibility Pattern of Isolates: In our study Gram negative isolates is overall 100% sensitive to Polymyxin , 90% sensitive to Levofloxacin ,76% sensitive to Imipenem, 61% sensitive to Piperacillin-Tazobactam, 47% sensitive to Minocycline, 45% sensitive Chlormphenicol, 43% sensitive to Amikacin and 38% sensitive to cefepime. As reported by P. jyothi *et al.* [15] Gram negative isolates overall is 93% sensitive to imipenem , 52% sensitive to Amikacin and 70% sensitive to levofloxacin . As reported by Srinivasa *et al.* [16] Gram negative isolates is overall 100% sensitive to Imipenem , 62% sensitive to levofloxacin, 33% sensitive to tetracycline and 62% sensitive to and cefepime. As reported by Maimoona Mustafa *et al*. [17] Gram negative isolates is overall 100% sensitive to Imipenem, 100% sensitive to colistin 63% sensitive to levofloxacin, 33% and 68% sensitive to Amikacin.

Pseudomonas Aeruginosa: In this study *Pseudomonas aeruginosa* 100% Sensitive to Polymyxin and levofloxacin, Amikacin, 75% sensitive to Aztreonam, Netilimycin and 50% sensitive to Imipenem, Piperacillin-Tazobactum, Ciprofloxacin. As reported by Madhu Sharma *et al*. [18] *Pseudomonas aeruginosa* is 62% sensitive to Imipenem, 20% sensitive to Amikacin, 12% sensitive gentamycin 10% sensitive to Piperacillin. As reported by Sanjay. D. Rathod *et al.* [9] *P. aeruginosa* showed less resistance than other isolated Gram negative bacilli. Of them 28.6% were resistant to ceftazidime and 14.3% to Aztreonam and 100% sensitive to Imipenem and Amikacin. As reported by Maimoona Mustafa *et al*. [17] *Pseudomonas aeruginosa* is 100% sensitive to Imipenem, colistine, 50% sensitive to Amikacin, Ciprofloxacin and 25% sensitive to Gentamycin.

ESBL Producing Isolates: Prevalence of Extended spectrum β-Lactamase producing organism in our study is 62% In *Klebsiella pneumonia* and 43% in *E. coli*. Other comparing study for ESBL producing isolates:

Study	Year	*Klebsiella pneumonia*	*E.coli*
Singh *et al.* [19]	2012	80%	60%
Bhattacharjee *et al.* [20]	2007	62.7%	46.5%
Ali Peirovifar *et al.* [21]	2012	86.7%	64.3%
Our study	2013	62%	43%

Here our study is comparable with Bhattacharjee *et al.* [20]

Amplified Cephalosporinase Producing Isolates: In our study Amp Cbetalactamase producing *Klebsiella pneumoniae* 15% and *E.coli* is 14%. For these comparing study as follow:

Study	Year	% AmpC Beta lactamase producer
Dhara Modi *et al.* [22]	2012	7.40
Chelliah *et al.* [23]	2011	6.1
our study	2013	15

So our study is comperable with Dhara Modi *et al.*

MBL Producer: Among other resistant patterns, prevalence of Carbapenemase producing *Klebsiella pneumoniae* was 19.23%, in *E. coli* 14.28% and in *Pseudomonas aeruginosa* MBL producer is 50 %.

According to other study as follow:

Study	Year	*Pseudomonas aeruginosa*
Prajapati Sweta *et al*. [24]	2013	44%
Madhu Sharma *et al*. [18]	2008	69.5%
Our study	2012	50%

So here our study is comparable with Prajapati Sweta. B et all.

CONCLUSION

Neonatal septicaemia is a life threatening emergency and rapid treatment with antibiotics is essential for a favourable outcome. Classical empirical treatment of neonatal sepsis consists of amoxicillin & an aminoglycoside. In present study, Gram-negative isolates were frequently found to be resistant to amoxicillin & aminoglycoside also, thus indicating that the use of these drugs might be ineffective. Therefore great caution is required in selection of antibiotic therapy. This highlights the variable nature of antibiotic susceptibility patterns both in time and location therefore; it is advisable to continuously evaluate the sensitivity-resistance pattern of isolates so as to make a rational use of antibiotics. These data support the hypothesis that determination of antibiotic sensitivity patterns in periodic intervals is mandatory in each region for choosing appropriate antibiotic therapy. In the view of above, the strategy of antibiotic usage in the hospital must be reviewed. The determination of various enzymes production by various bacterial isolates like beta-lactamase, ESBL, Amp-c betalactamase will help us to identify drug resistance pattern.

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