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Research Article

The Changing Bacteriological Profile with their Antibiograms and Outcome of Culture Positive Neonatal Sepsis in a Tertiary Care Centre of Eastern India

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Received: January 12, 2018; **Published:** March 02, 2018

Abstract

Introduction: Sepsis is one of the most important causes of neonatal mortality. During sepsis, upregulated cytokines and pro-inflammatory cells result in brain damage among both term and preterm neonates. However, the microbial spectrum and their antibiograms show inter-regional variation and even vary in hospitals of the same region.

Methods and Materials: The study was conducted at Dr. B C Roy Post Graduate Institute of Pediatric Sciences from July 2015 to June 2017, the period of 2 years being divided into 4 phases of 6 months each. The study included all septic neonates (positive blood culture by BACTEC method), admitted during the study period. The blood culture reports were retrospectively analyzed. The neonates were followed up at the high risk clinic till 1 year of age; their neuromotor examination, developmental assessment, and electrophysiological investigations were done. The trend of changing bacteriological profiles and antibiograms of the 3 most common isolates were analysed. The risk factors of mortality and morbidity were analysed using univariate and multivariate analysis. Their odds and risk ratios with their 95% confidence intervals were calculated.

Results: 556 neonates had positive blood culture. The mean (± standard deviation) gestational age and weight at presentation were 36.2 ± 6.2 weeks and 2.7 ± 1.2 kg. Lethargy with poor feeding was the most common (60%) presentation. 44.96% were preterm, 53.94% had low birth weight and 51.62% had early onset sepsis. 13.31% had meningitis. *Klebsiella* species (42.45%), *Staphylococcus aureus* (27.88%) and coagulase negative *Staphylococcus* (11.51%) were the 3 most common isolates. Sensitivity to several antibiotics showed significant changes over the 4 phases. Mortality and morbidity was 40.54% and 68.18% among neonates with meningitis and it was significantly lower, 8.29% and 24.69% ($p < 0.0001$), among neonates with sepsis without meningitis. Incidence of microcephaly, and abnormalities in electrophysiological studies were significantly higher among neonates with developmental delay. Spontaneous preterm labour [$p = 0.0018$, OR = 2.2 (1.3 - 3.8)], deliveries at < 37 weeks gestation [$p < 0.0001$, OR = 3.1 (1.8 - 5.2)], preterm rupture of membrane [$p = 0.0035$, OR = 2.1 (1.2-3.5)] and meningitis [$p < 0.0001$, OR = 7.5 (4.2 - 13.3)] were the significant risk factors for mortality. Multivariate analysis showed prematurity, low birth weight and organ dysfunction to be the independent predictors of developmental delay.

Conclusion: *Klebsiella* species is still the predominant organism of neonatal sepsis in the Indian subcontinent, although significant rise in proportion of *Staphylococcus aureus* is occurring. Strategies aimed at decreasing the incidence of prematurity would eventually reduce morbidity and mortality in neonatal sepsis. Since organ dysfunction strongly predicted poor neurodevelopmental outcome, close monitoring and earlier initiation of aggressive therapy in intensive care units could help in improving outcome.

Keywords: *Neonatal Sepsis; Meningitis; Klebsiella Species; Developmental Assessment Scale for Indian Infants*

Introduction

Neonatal sepsis is the systemic infection of the newborn, characterized by nonspecific symptoms, and documented by positive blood culture [1]. An estimated 1.6 million deaths occur due to neonatal infections worldwide, 40% being from developing countries [2]. An Indian multicentric study implicated sepsis as one of the most important causes of mortality, contributing to 19% of all neonatal deaths. The incidence of neonatal sepsis in India varies from 11 - 24.5/1000 live births [3]. During inflammation, there is a systemic up-regulation of pro-inflammatory cytokines and diffuse activation of microglia in the neonatal brain. Microglia enhances injury by expressing inflammatory mediators and pro-inflammatory cytokines [tumor necrosis factor, interleukin-1, interleukin-6, and interleukin-8], reactive oxygen species and toxic granules including proteolytic enzymes and myeloperoxidase [4]. The pro-inflammatory cytokines can activate cytotoxic T cells, natural killer cells, lymphokine-activated killer cells, which enhance excessive cellular and tissue damage [4]. This results in cell proliferation, cell differentiation, and cell death, all causing white matter damage (WMD) and long-term neurological injury among preterm and term neonates and hence, this topic assumes paramount significance [5].

The microbial spectrum of neonatal sepsis shows inter-regional variation and even varies in hospitals of the same region. In addition, one or a group of organisms may be replaced by others over a period of time [6]. In developed countries, Gram-negative organisms were replaced by Group B *Streptococcus* (GBS) and coagulase-negative *Staphylococcus* (CONS) species in 70s as the most common organisms of neonatal sepsis [7,8]. In India, gram-negative bacteria till recently were reported to be the major cause of neonatal sepsis with predominance of *Klebsiella pneumonia*, although the proportion of Gram-positive bacteria, especially *Staphylococcus aureus*, has gradually increased over the last two decades [9,10]. According to the Indian National Neonatal Perinatal Database, *Klebsiella pneumoniae, Staphylococcus aureus* and *E. coli* are the three most common organisms causing neonatal sepsis both in hospital and community [11]. Sepsis occurring in the first 72 hours of life is defined as early-onset sepsis (EOS) and that occurring beyond 72 hours as late-onset sepsis (LOS). The causative organisms of EOS and LOS sepsis are similar especially in hospital settings in developing countries [11]. Knowledge of the common pathogens causing septicemia in neonates and their antimicrobial susceptibility is essential in order to select appropriate antimicrobial treatment. Antimicrobial susceptibility patterns vary geographically and are temporally dependent on local pathogens and patterns of antibiotic use in a particular neonatal unit [11]. The widespread emergence of resistance to multiple commonly used antibiotics is challenging for determining appropriate empirical therapy. This varying microbiological pattern of neonatal septicaemia warrants an ongoing review of the causative organisms and their antimicrobial susceptibility pattern.

Thus, the present study aimed to know the trend of neonatal sepsis organisms and their antibiotic susceptibility pattern over 2 years, and identify factors associated with poor neurodevelopmental outcome in order to institute preventive strategies that will decrease morbidity and mortality.

Methods and Materials

Study settings

This study was conducted after institutional ethics committee approval at the departments of Microbiology and Pediatrics at a tertiary care pediatric hospital in Kolkata. Informed written consent was taken from parents prior to inclusion of the neonates in the study. The institute has 150 bedded sick newborn care unit and 20 bedded neonatal intensive care unit and admits only extramural newborns. A retrospective analysis of blood cultures of all cases of neonatal septicemia during the past 2 years were done and divided in four phases: July 2015 to December 2015 (Phase I), January 2016 to June 2016 (Phase II), July 2016 to December 2016 (Phase III), January 2017 to June 2017 (Phase IV).

Inclusion criteria

Only institutional delivered infants were included in the study. Centers for Disease Control/National Healthcare Safety Network criteria were used for assessment of bloodstream infections [12]. We included those newborns with maternal risk factors for presumed sepsis such as fever, premature rupture of membrane > 24 hrs, > 2 unclean or clean per vaginal examinations, instrumental delivery. Also newborns with clinical signs and symptoms of sepsis were included. Clinical signs included worsening of respiratory distress: tachypnea, sternal and/or subcostal retraction, groaning and cyanosis, apnea, body temperature instability, hyper- or hypoglycemia, poor peripheral perfusion, food intolerance, arterial hypotension, and underactive infants.

Exclusion criteria

Neonates with birth asphyxia, hyperbilirubinemia requiring exchange transfusion, and major congenital malformations, as they might be confounding factors in evaluating the neurologic outcome.

Method

Parents filled a structured questionnaire regarding the medical, surgical and obstetric history to identify and assess the risk factors. For every infant included in the study, sepsis screen (blood counts with absolute neutrophil count, peripheral smear for band cell to neutrophil ratio and toxic granulations, C-reactive protein, and micro-ESR), and blood cultures were sent. Cerebrospinal fluid (CSF) examination was done for neonates with suspected meningitis. Blood sugar, electrolytes, and arterial blood gas parameters were periodically monitored; liver and renal function tests, coagulation profile were done when deemed necessary.

Blood cultures from neonates having suspected sepsis were collected before starting empirical antibiotics, as per hospital protocol. The local site was cleansed with 70% alcohol and povidone iodine (1%) followed by 70% alcohol again. Under stringent aseptic conditions, 1 ml of blood was collected and inoculated into 20 ml BacT alert blood culture bottle and incubated at 37°C for 7 days. Subcultures were done after 1, 2, 3, 5, and 7 days on blood agar and MacConkey agar and incubated aerobically overnight to 48 hours at 37°C. All positive cultures were identified by their characteristic appearance on the respective media, gram staining and pattern of biochemical reactions using standard methods. Coagulase negative *Staphylococcus* (CONS) was considered a pathogen only when isolated in paired cultures. Growth of mixed bacterial flora or diphtheroids was considered as contamination. Bacterial isolates were identified and antibiotic susceptibility test was performed using VITEK 2 automated antibiotic susceptibility testing (AST) system (bioMérieux India Private Limited, New Delhi). After primary organism isolation, handling was minimized in a simple standardized inoculum into the VITEK 2 Cassette, where the VITEK 2 Card and the sample are linked virtually and susceptibility results were provided in as little as 5 hours.

Follow-up

After discharge, the newborns were followed up at the outpatient high-risk clinic at 1, 3, 6, 9, 12 months corrected age. Clinical and neurological assessment was performed by a pediatrician and a physical therapist monthly until 12 months of age. This consisted of the observation of spontaneous movements and posture, muscle tone, asymmetries and reflexes according to the Amiel-Tison protocol, and development milestones according to Denver Development Screening Test (DDST). Neuromotor impairment was considered when the child showed change in muscle tone, abnormal posture, abnormal spontaneous movements, altered neurological examination, and motor or mental developmental delay. Infants with neuromotor delay were definitively evaluated by Developmental Assessment Scale for Indian Infants (DASII) to calculate the motor (MoDQ) and mental developmental (MeDQ) quotients, where DQ < 70 signifies developmental delay.

Electroencephalogram (EEG), visual evoked potential (VEP) and brainstem auditory evoked potential (BAER) tests were done for each infant.

Statistical analysis

The data obtained from the questionnaire and laboratory investigations were analyzed using EPI-INFO 36.1software version 2008 and Statistical Package for the Social Sciences (SPSS) version 21 by cross tabulation of risk factors and univariate/multivariate analysis. The odds and risk ratios with their 95% confidence intervals were calculated. The p-values were calculated using Fisher's exact test (for categorical variables) and Student's t-test (for normally distributed variables) and value < 0.05 was considered significant.

Result

Total number of neonatal admission during the study period was 4532.

556 (12.26%) neonates had positive blood cultures.

349 (62.77%) were males and 207 (37.23%) were females.

306 (55.04%) were term neonates and 250 (44.96%) were preterm neonates. In 240 cases there was spontaneous preterm labour and in 10 cases labour was induced due to maternal complications like gestational hypertension, diabetes and placental abruption.

256 (46.04%) had birth weight > 2.5 kg and 300 (53.94%) had low birth weight (LBW) < 2.5 kg.

287 (51.62%) were early onset sepsis (EOS) and 269 (48.38%) were late onset sepsis (LOS).

The mean (± standard deviation) gestational age and weight at presentation were 36.2 ± 6.2 weeks and 2.7 ± 1.2 kg.

The neonates presented with lethargy and poor feeding [333 (60%)], hypothermia [222 (40%)], fever [55 (10%)], sclerema [195 (35%)], foul umbilical discharge [50 (9%)], convulsion [111 (20%)], abdominal distension [100 (18%)], exaggerated physiological hyperbilirubinaemia [167 (30%)], pustules [28 (5%)], and bleeding manifestations [44 (8%)]. 74 (13.31%) patients had meningitis.

Causative organisms

324 (58.27%) had Gram-negative sepsis and 232 (41.73%) had Gram-positive sepsis. *Klebsiella* species (42.45%), *Staphylococcus aureus* (27.88%) and CONS (11.51%) were the 3 most common isolates. Table 1 shows the incidences of the causative organisms of EOS and LOS and the lack of significant differences among them. Among 74 neonates with meningitis, 31 (41.89%) had gram positive sepsis and 43 (58.11%) had gram negative sepsis (p = 0.081).

Organisms	Total no. (%) (n = 556)	EOS (n = 287)	LOS (n = 269)
Gram negative organisms	**324**	**172**	**152**
Klebsiella sp.	236 (42.45%)	125	111
E.coli	20 (3.59%)	16	4
Acinetobacter	20 (3.59%)	9	11
Pseudomonas	15 (2.69%)	7	8
Enterobacter	7 (1.26%)	3	4
Salmonella	7 (1.26%)	4	3
Citrobacter	6 (1.08%)	2	4
Serratia	6 (1.08%)	3	3
Kokuria	4 (0.72%)	2	2
Sphingomonas	2 (0.36%)	1	1
Elizabethkingia	1 (0.18%)	0	1
Gram positive organisms	**232**	**115**	**117**
Staphylococcus aureus	155 (27.88%)	76	79
CONS	64 (11.51%)	31	33
Enterococcus	13 (2.33%)	8	5

Table 1: *Shows the incidences of the Gram-negative and Gram-positive organisms of EOS and LOS.*

Over the 2 years study period, *Klebsiella* sp. remained the predominant organism in phases I, II and IV, although with decreasing relative incidence- 58.3%, 43.2% and 35.2% respectively, as shown in table 2. *Staphylococcus aureus* was the predominant organism (35.6%) in phase III. There was significant increase in the incidence of CONS and other organisms (comprising *Pseudomonas aeruginosa*, *Enterococcus* species, *Salmonella* species, *Enterobacter* species, *Serratia* species, *Citrobacter* species, *Kocuria kristinae*, *Sphingomonas paucimobilis* and *Elizabethkingia meningoseptica*) in phase IV.

Organism	Phase I (1.7.15 to 0.12.15), n = 139	Phase II (1.1.16 to 0.6.16), n = 146	Phase III (1.7.16 to 0.12.16), n = 115	Phase IV (1.1.17 to 30.6.17), n = 156	p-value for trend
Klebsiella pneumoniae	81 (58.3%)	63 (43.2%)	36 (31.3%)	56 (35.89%)	< 0.0001
Staphylococcus aureus	35 (25.7%)	44 (30.1%)	41 (35.6%)	35 (22.4%)	< 0.0001
Coagulase -ve staphylococcus	3 (2.1%)	16 (10.9%)	13 (2.6%)	32 (20.5%)	0.242
E.coli	6 (4.3%)	7 (4.8%)	7 (6%)	0	0.0041
Acinetobacter baumanii	6 (4.3%)	6 (4.1%)	3 (2.6%)	5 (3.1%)	0.25428
Others	8 (5.7%)	10 (6.8%)	15 (13%)	28 (18%)	< 0.0001

Table 2: *Shows the changing trend of the causative organisms of neonatal sepsis over the 4 phases.*

Antibiograms

Table 3a shows that *Staphylococcus aureus* isolates exhibited maximum sensitivity to linezolid ranging from 82.9 - 100%. Teicoplanin had uniformly good sensitivity between 82.9 - 88.6%. There were no significant changes in the sensitivity to linezolid, teicoplanin, vancomycin, gentamicin and ciprofloxacin. However, the sensitivity to tigecycline significantly raised from 20% in phase I to 82.8% in phase IV and sensitivity of amoxicillin-clavulanate significantly declined from 40% in phase I to 5.7% in phase IV. CONS growths were uniformly susceptible to linezolid in all the 4 phases as shown in table 3b. Although the isolates were uniformly susceptible to vancomycin in phases I and II, sensitivity significantly decreased to 69.2% in phase III, but thereafter again increased to 87.5% in phase IV. Tigecycline sensitivity also significantly reduced from 100% in phases I and II to 92.3% and 81.2% in phases III and IV. Significant numbers of isolates were sensitive to gentamicin in phase II (62.5%) and phase III (69.2%) and to ciprofloxacin in phase IV (21.9%).

Antibiotic	Phase I (n = 35)	Phase II (n = 44)	Phase III (n = 41)	Phase IV (n = 35)	p-value for trend
Linezolid	35 (100%)	40 (90.9%)	34 (82.9%)	33 (94.3%)	0.34212
Vancomycin	34 (97.1%)	38 (36.4%)	27 (65.9%)	25 (71.4%)	0.05744
Teicoplanin	31 (88.6%)	37 (84%)	34 (82.9%)	30 (85.7%)	0.32218
Tigecycline	7 (20%)	34 (77.3%)	31 (75.6%)	29 (82.8%)	< 0.0001
Coamoxyclav	14 (40%)	0	0	2 (5.7%)	< 0.0001
Gentamicin	20 (57.1%)	24 (54.5%)	23 (56%)	20 (57.1%)	0.48392
Ciprofloxacin	18 (51.4%)	21 (47.7%)	18 (43.9%)	15 (42.8%)	0.25014

Table 3a: *Shows the sensitivity trend of Staphylococcus aureus over the 4 phases.*
In phase I, 14 received coamoxyclav, 12 received vancomycin, 5 received gentamicin, and 4 received linezolid; in phase II, 24 received gentamicin, 10 received linezolid, 5 each received vancomycin and ciprofloxacin; in phase III, 23 received gentamicin, 10 received ciprofloxacin, 6 received vancomycin and 2 received linezolid; in phase IV, 20 received gentamicin, 6 received linezolid, 5 received ciprofloxacin and 4 received vancomycin.

Antibiotic	Phase I (n = 3)	Phase II (n = 16)	Phase III (n = 13)	Phase IV (n = 32)	p-value for trend
Linezolid	3 (100%)	16 (100%)	13 (100%)	32 (100%)	0.52
Vancomycin	3 (100%)	16 (100%)	9 (69.2%)	28 (87.5%)	< 0.0001
Teicoplanin	3 (100%)	16 (100%)	11 (84.6%)	25 (78.1%)	< 0.0001
Tigecycline	3 (100%)	16 (100%)	12 (92.3%)	26 (81.2%)	< 0.0001
Coamoxyclav	1(33.3%)	0	0	2 (6.25%)	0.41222
Gentamicin	1(33.3%)	10 (62.5%)	9 (69.2%)	9 (28.1%)	0.00262
Ciprofloxacin	0	0	0	7 (21.9%)	0.00018
Oxacillin	1(33.3%)	0	2 (15.4%)	4 (12.5%)	0.09492

Table 3b: *Shows the sensitivity trend of CONS over the 4 phases.*
In phase I, 1 received coamoxyclav and 2 received linezolid; in phase II, 9 received gentamicin, 4 received linezolid, and 3 received vancomycin; in phase III, 9 received gentamicin, and 2 each received vancomycin and linezolid; in phase IV, 11 received linezolid, 9 received gentamicin, 6 received vancomycin, 4 received ciprofloxacin, and 1 each received coamoxyclav and tigecycline.

Table 3c shows that the sensitivity of Klebsiella sp. isolates significantly reduced to amikacin and gentamicin from phase I (87.6% and 85%) to phase IV (35.7% and 19.6%). Sensitivity was uniformly high (> 90%) to tigecycline and colistin. Significant reduction in sensitivity was noted for piperacillin-tazobactum, ciprofloxacin and co-amoxyclav, from 28.4%, 85.2% and 25.7% in phase I to 10.7%, 26.8%, 10.7%, in phase IV. However, sensitivity to meropenem significantly increased from 23.4% in phase I to 42.8% in phase IV.

Antibiotic	Phase I (n = 81)	Phase II (n = 63)	Phase III (n = 36)	Phase IV (n = 56)	p-value for trend
Amikacin	71 (87.6%)	55 (87.3%)	32 (88.9%)	20 (35.7%)	< 0.0001
Gentamicin	69 (85%)	45 (71.4%)	18 (50%)	11 (19.6%)	< 0.0001
Meropenem	19 (23.4%)	35 (55.6%)	18 (50%)	24 (42.8%)	0.00614
Colistin	81 (100%)	61 (96.8%)	33 (91.6%)	53 (94.6%)	< 0.0001
Tigecycline	81 (100%)_	63 (100%)	36 (100%)	54 (94.6%)	< 0.0001
Cefepime	23 (28.4%)	16 (25.4%)	7 (19.4%)	8 (14.2%)	0.00058
Cefoperazone+sulbactam	23 (28.4%)	17 (26.9%)	11 (30.6%)	21 (37.5%)	0.01878
Ciprofloxacin	69 (85.2%)	44 (69.8%)	18 (50%)	15 (26.8%)	< 0.0001
Piprecillin+tazobactam	23 (28.4%)	13 (20.6%)	4 (11.1%)	6 (10.7%)	< 0.0001
Coamoxyclav	20 (24.7%)	11 (17.4%)	4 (11.1%)	6 (10.7%)	0.0001

Table 3c: *Shows the sensitivity trend of Klebsiella isolates over the 4 phases.*
In phase I, 71 received amikacin, 5 received meropenem, 3 received ciprofloxacin and 2 received colostin; in phase II, 55 received amikacin, 4 received piperacillin-tazobactum, 3 received meropenem and 1 received colistin; in phase III, 32 received amikacin, 2 received meropenem and 1 each received colistin and tigecycline; in phase IV, 20 received amikacin, 19 received meropenem, 5 each received colistin, ciprofloxacin and cefoperazone-sulbactum and 2 received tigecycline.

Antibiotics	*Klebsiella* (n = 236)	*E coli* (n = 20)	*Acinetobacter* (n = 20)	*Pseudomonas* (n = 15)	*Enterobacter* (n = 7)	*Salmonella* (n = 7)	*Citrobacter* (n = 6)	*Serratia* (n = 6)
Cefotaxime	20 (8.45%)	2 (10%)	0	1 (6.67%)	2 (28.57%)	2 (28.57%)	3 (50%)	2 (33.33%)
Cefepime	54 (22.88%)	7 (35%)	2 (10%)	3 (20%)	5 (71.42%)	5 (71.42%)	5 (83.33%)	4 (83.33%)
Amikacin	178 (75.42%)	15 (75%)	8 (40%)	10 (66.67%)	6 (85.71%)	6 (85.71%)	6 (100%)	6 (100%)
Gentamycin	143 (60.59%)	13 (65%)	2 (10%)	7 (46.67%)	6 (85.71%)	4 (57.14%)	5 (83.33%)	4 (83.33%)
Piperacillin	46 (19.49%)	13 (65%)	5 (25%)	9 (60%)	6 (85.71%)	4 (57.14%)	5 (83.33%)	5 (83.33%)
Ciprofloxacin	146 (61.86%)	14 (70%)	10 (50%)	6 (40%)	5 (71.42%)	6 (85.71%)	5 (83.33%)	4 (66.67%)
Meropenem	96 (40.67%)	16 (80%)	12 (60%)	9 (60%)	7 (100%)	7 (100%)	6 (100%)	6 (100%)
Colistin	234 (99.15%)	20 (100%)	18 (90%)	15 (100%)	7 (100%)	7 (100%)	6 (100%)	6 (100%)

Table 3d: *Shows the antibiotic sensitivity pattern of other Gram negative organisms.*

Mortality

70 (12.59%) neonates died. Among 74 neonates with meningitis, 30 (40.54%) died. Analysis of risk factors in table 4 showed that spontaneous preterm labour (p = 0.0018, OR = 2.2), deliveries at < 37 weeks gestation (p < 0.0001, OR = 3.1), premature rupture of membrane (p = 0.0035, OR = 2.1), and meningitis (p < 0.0001, OR = 7.5) were the significant risk factors for mortality.

Characteristics	Survivors (n = 486)	Death (70)	Odds ratio (95% CI)	p value
Antenatal corticosteroid therapy	60	10	0.84 (0.41-1.73)	0.646
PROM	218	44	2.1 (1.2-3.5)	0.0035
Spontaneous preterm labour	240	48	2.2 (1.3-3.8)	0.0018
Singleton pregnancy Twin pregnancy	480 6	68 2	2.4 (0.5-11.9)	0.2661
BOH	19	5	0.5 (0.1-1.4)	0.1723
Maternal illness during pregnancy	16	4	0.6 (0.2-1.7)	0.2361
Sex Male Female	301 185	48 22	0.7 (0.4-1.2)	0.1734
Gestational age < 37 weeks > 37 weeks	202 284	48 22	3.1 (1.8-5.2)	< 0.0001
Duration of central venous lines >10 days <10 days	333 153	61 9	0.3 (0.2-0.7)	0.0018
Birth weight <2.5 kg >2.5 kg	260 226	40 30	0.9 (0.5-1.4)	0.3294
EOS LOS	250 236	37 33	0.9 (0.5-1.6)	0.8984
Gram -ve sepsis Gram +ve sepsis	288 198	36 34	0.727 (0.44-1.203)	0.214
Meningitis No meningitis	44 442	30 40	7.5 (4.2-13.3)	< 0.0001

Table 4: *Enumerates the risk factors for mortality.*

Morbidity

Among 486 survivors, 16 were lost to follow-up and hence, 470 neonates were followed upto 1 year of age. 295 (62.76%) were males and 175 (37.24%) were females. 250 (53.19%) were LBW and 220 (46.81%) were > 2.5 kg. 270 (57.45%) were term neonates and 200 (42.55%) were preterm. 228 (48.51%) being EOS and 242 (51.49%) being LOS. 335 (75.72%) had normal outcome and 135 (24.28%) had developmental delay. Among the 44 survivors of meningitis, 30 (68.18%) had developmental delay. 4 infants with meningitis developed hydrocephalus, 2 needed ventriculo-peritoneal shunt and 2 had arrested hydrocephalus. Table 5 shows the developmental quotients and the incidences of microcephaly, abnormal BAER, VEP and seizure disorder among the survivors.

Characteristics	Normal outcome (n = 335)	Developmental delay (n = 135)
Microcephaly	15 (4.47%)	70 (51.85%)
Abnormal BAER	10 (2.95%)	50 (37.03%)
Abnormal VEP	0	20
Seizure disorder	10 (2.95%)	40 (29.63%)
Mean(±SD) MoDQ	90.4 ± 4.6	54.55 ± 15.78
Mean(±SD) MeDQ	89.2 ± 3.5	49.98 ± 12.75

Table 5: *Shows the outcome of the survivors of culture positive neonatal sepsis.*

Table 6a shows that low birth weight < 2.5 kg [OR = 1.53 (CI 1.02 - 2.3), p = 0.02], platelet < 50,000 [OR = 1.97 (CI 1.31 - 2.97), p = 0.0007], presence of renal failure [OR = 1.5 (CI 1.2 - 2.19), p = 0.03], coagulopathy [OR = 4.88 (CI 2.22 - 4.75), p = <0.0001], hypoglycemia [OR = 6.41 (CI 4.94 - 8.92), p = < 0.0001], and hypocalcemia [OR = 1.71 (CI 1.14 - 2.56), p = 0.006], need for mechanical ventilation [OR = 13.51 (CI 7.95 - 22.94), p < 0.0001], occurrence of meningitis [OR = 6.55 (CI-3.34 - 12.82), p < 0.0001] and gram negative sepsis [OR = 0.39 (CI 0.25 - 0.59), p < 0.0001] were the independent risk factors for poor outcome. Thus, need of ventilation [for apnea, hypercapnea ($PaCO_2$ > 60 mm Hg) or hypoxemia (PaO_2 < 80 mm Hg)] was the strongest predictor for poor outcome; ventilated infants were 13 times more likely to have poor neurodevelopmental outcome. When entered into a multivariate logistic regression model (Table 6b), low birth weight, prematurity, hypoglycaemia, hypocalcemia, thrombocytopenia, acute kidney injury, coagulopathy, septic shock, need for mechanical ventilation and meningitis were the predictors of poor outcome.

Characteristics	Normal outcome (n = 335)	Delay (n = 135)	OR (95% CI)	RR (95% CI)	p value
Early onset sepsis Late onset sepsis	170 165	58 77	0.73 (0.48-1.89)	0.79 (0.59-1.06)	0.07
Birth weight < 2.5 kg > 2.5 kg	 168 167	 82 53	1.53 (1.02-2.30)	1.36 (1.01-1.82)	0.02
Term Preterm	205 130	65 70	0.6(0.4-0.9)	0.7 (0.5-0.9)	0.006
Sex : Male Female	202 133	93 42	1.45 (0.95-2.23)	1.31 (0.96-1.79)	0.05
TLC < 4000 TLC > 4000	204 131	78 57	0.87 (0.58-1.31)	0.91 (0.68-1.21)	0.3
Platelet < 50,000 Platelet > 50,000	155 180	85 50	1.97 (1.31-2.97)	1.62 (1.20-2.19)	0.0007
Acute kidney injury	140	70	1.5 (1.00-2.24)	1.33 (1.00-1.77)	0.03
Coagulopathy	102	92	4.88 (3.17-7.51)	3.04 (2.22-4.15)	<0.0001
Hypoglycaemia	72	86	6.41 (4.14-8.92)	3.46 (2.58-4.65)	<0.0001
Hyponatremia	148	64	1.13 (0.76-1.70)	1.09 (0.82-1.48)	0.29
Hypocalcemia	120	66	1.71 (1.14-2.56)	1.46 (1.10-1.93)	0.006
Septic shock	79	44	1.56 (1.009-2.431)	1.38 (0.9-1.92)	0.049
Need for mechanical ventilation	100	115	13.51 (7.95-22.94)	6.81 (4.39-10.57)	<0.0001
Gram –ve sepsis Gram +ve sepsis	208 143	80 55	1 (0.66-1.49)	1 (0.74-1.34)	1
Meningitis No meningitis	14 321	30 105	6.551 (3.34-12.82)	2.76 (2.13-3.59)	<0.0001

Table 6a: *Enumerates the risk factors of poor outcome.*

Characteristics	β	SE	Wald chi square	Exponential of β (95% CI)	P
Prematurity	0.086	0.018	22.82728	1.54 (0.98-2.45)	0.0412
Birth weight	2.063	0.798	6.68331	1.53 (1.02-2.30)	0.02
Thrombocytopenia	3.010	0.889	11.4638	1.97 (1.31-2.97)	0.0007
Acute kidney injury	3.111	0.798	6.1632	1.5 (1.00-2.24)	0.03
Coagulopathy	4.156	0.928	14.874	4.88 (3.17-7.51)	< 0.0001
Hypoglycemia	-2.132	0.766	7.7467	6.41 (4.14-8.92)	< 0.0001
Septic shock	3.214	0.697	21.26307	1.56 (1.009-2.431)	0.049
Need for mechanical ventilation	-2.101	0.797	6.94923	13.51 (7.95-22.94)	< 0.0001
Meningitis	-2.010	0.804	6.25	6.551 (3.34-12.82)	< 0.0001

Table 6b: *Shows the multiple logistic regression analysis for the risk factors of poor outcome. [β: Beta, SE: Standard Error]*

Discussion

Discussion on causative organisms

In this study, gram-negative organisms predominate probably because newborns acquire these gram-negative rods from the vaginal and fecal flora of the mother and the environment where the delivery occurs [13]. Importance of both vertical transmission from the mother and postnatal acquisition of infection from the environment has been suggested in the literatures for pathogenesis of neonatal sepsis [14]. Recently, *Staphylococci* are emerging as the most common cause of neonatal septicemia, the main source being the hands of health-care providers in the heavily contaminated environment to which mother and child are exposed during labor, delivery, and post-natal care [2,15,16]. Table 7 shows the comparison of the common isolates among different studies. The West Indies studies and all the Indian studies except Dalal [17] and Kartikeyan [18] found *Klebsiella* as the most predominant organism; however, the French study [19] found GBS to be predominant. Dalal [17] reported *Pseudomonas* (47.58%) and Kartikeyan [18] reported *Staphylococcus* (61.5%) to be the most common organisms in their studies. Another Indian study [20] from South India interestingly reported *Burkholderia cepacia* (30%) as the most common isolate in their study followed by *Klebsiella* (15.5%) and *Staphylococcus aureus* (14.7%). In India, colonisation rates in infants born to asymptomatic maternal carriers of GBS are 53-56%, which is consistent with rates reported from other parts of the world. Despite significant GBS colonisation rates, invasive neonatal GBS disease in India is infrequent. During a 10-year study between 1988 and 1997, in a tertiary care perinatal centre in south India, the incidence of neonatal GBS infection was 0.17 per 1000 live births [21].

Studies	*Klebsiella*	*E. coli*	*Acinetobacter*	*Pseudomonas*	*S. aureus*	*CONS*	*GBS*	Others
Index study (Kolkata, East India)	42.45	3.59	3.59	2.69	27.88	11.51	-	7.29
Premlatha (Karnataka, South India) [11]	38.1	4.8	7.1	4.76	2.4	28.6	-	19
Raj C (Andhra Pradesh, South India) [22]	36.2	21.4	3.9		10.7	7.4	-	18.6
Jayasimha (Karnataka, South India) [3]	21.6	7.5	14.4	13.75	20	12.5	-	24.4
Dalal (Haryana, North India) [17]	4.21	12.35	15.35	47.58	12.35	4.2	-	61.54
Mythri (Karnataka, South India) [23]	64.6	5.2	-	4.59	9.1	53.5	-	27.3
Samaga (Karnataka, South India) [24]	60.7	7.1	-	-	14.3	-	-	17.9
Karthikeyan (Chennai, South India) [18]	21.9	13.5			61.5		3.1	-
Shah (Gujarat,West India) [14]	12	20	7	10	13	27	-	23
Shobowale (West Indies) [25]	40.0	-	4.7	-	18.8	11.8	-	24.7
Trotman (West Indies) [26]	27.58	16.55	1.37	2.75	8.27	8.96	10.34	26.93
Mitha (France) [19]		33			20		34	-

Table 7: *Shows the comparison of the isolates (%) in various Indian and foreign studies.*

Discussion on antibiograms

Very few studies evaluated the antibiotic sensitivity pattern over a period of time. Approximately 21% of *staphylococcus isolates* were methicillin sensitive. Both Staphylococcus aureus and CONS exhibited high degree of sensitivity to linezolid and it did not change significantly over the study period. However, CONS isolates had a significant decline in vancomycin sensitivity in phase III, possibly due to its widespread use for methicillin resistant isolates. The sensitivity again increased in phase IV, possibly due to switching over to other drugs like gentamicin, teicoplanin and linezolid, which had high sensitivity in phase III. Most of the previous Indian studies documented methicillin resistance in the range of 25% - 40%, and Roy [16] and Karthikeyan [18] reported even higher resistance upto 47.4% and 66%. The use of glycopeptides and linezolid has been encouraged for such cases. Raj [22] and Samaga [24] reported 100% sensitivity to vancomycin and linezolid, whereas Dalal [17] reported 91% and 82% sensitivity respectively. The sensitivity of *Staphylococcus aureus* to amoxicillin-clavulanate was 30% (Dalal) [17] and 27.3% (Samaga) [24], compared to 22.85% in our study, and of CONS was 27% (Dalal) [17] and 25% (Samaga) [24], compared to 19.77% in our study.

Table 3c showed that *Klebsiella* isolates had significant decline in sensitivity to 3rd and 4th generation cephalosporins (cefoperazone and cefepime), aminoglycosides (amikacin and gentamicin), fluoroquinolones (ciprofloxacin) and amino-penicillin (piperacillin) over the study period. These multi-resistant strains were treated with reserve drug colistin, whose widespread use lead to decline in sensitivity from 100% in phase I to 94.6% in phase IV. However, meropenem which had low degree of sensitivity (23.4%) in phase I, exhibited a significant increase in sensitivity to 42.8% in phase IV and was helpful in treating multi-resistant strains. Roy [16] attributed some change in antibiotic resistance to the frequency of the use of particular antibiotics. In his study, widely used amikacin demonstrated a steady resistance across the study period. Infrequently used netilmicin saw a drop in resistance over the years. Indian studies reported moderately high degree of sensitivity of *Klebsiella* to amikacin and piperacillin-tazobactum - 69% and 80% (Jayasimha) [3], 82.3% and 94.1% (Samaga) [24], and 73% and 93% (Dalal) [17]. In our study the average sensitivity to amikacin was 75.42%, but it was low (19.49%) to piperacillin-tazobactum. Indian studies reported low sensitivity to traditionally used cefotaxime- 51% (Jayasimha) [3] and 53% (Samaga) [24]. Sensitivity to cefepime was 22.88% which was comparable to 27% sensitivity in Dalal's [17] study. However, Dalal reported higher sensitivity to carbapenems (87%) compared to 40.67% in our study. Shah [14] described 100% sensitivity to cefoperazone-sulbactam as compared to low average sensitivity of 30.85% in our study.

Figures 1a, 1b, 1c shows the comparison of antibiotic sensitivity of *E. coli, Acinetobacter* and *Pseudomonas* species among various Indian studies and since, the majority of them reported moderately high sensitivity to amikacin, carbapenems and piperacillin, we recommend the combination of amikacin with either carbapenems or piperacillin as the empirical therapy.

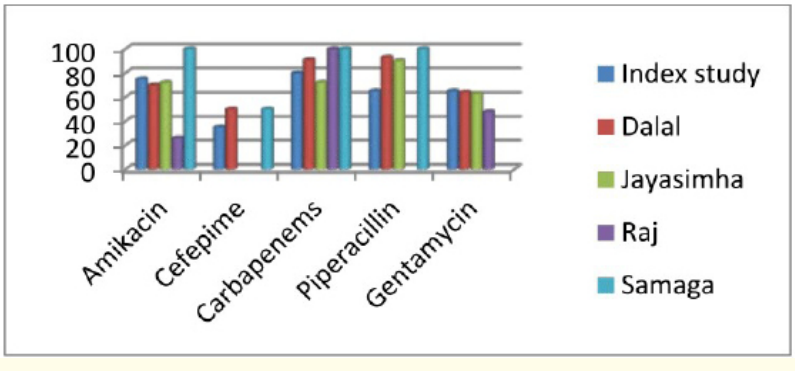

Figure 1a: *Shows the comparison of antibiotic sensitivity of E.coli among various Indian studies.*

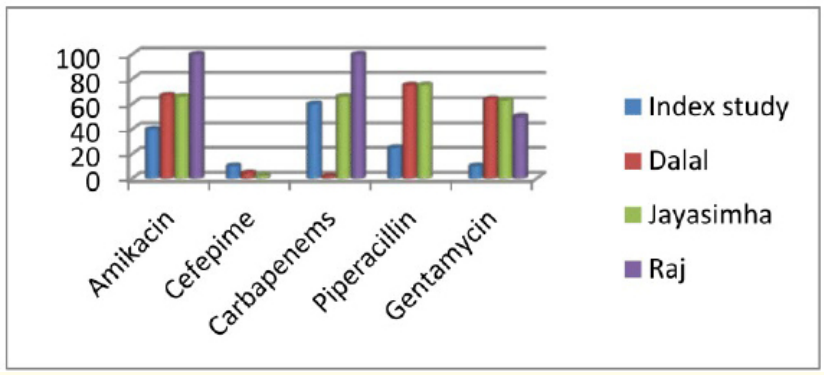

Figure 1b: *Shows the comparison of antibiotic sensitivity of Acinetobacter species among various Indian studies.*

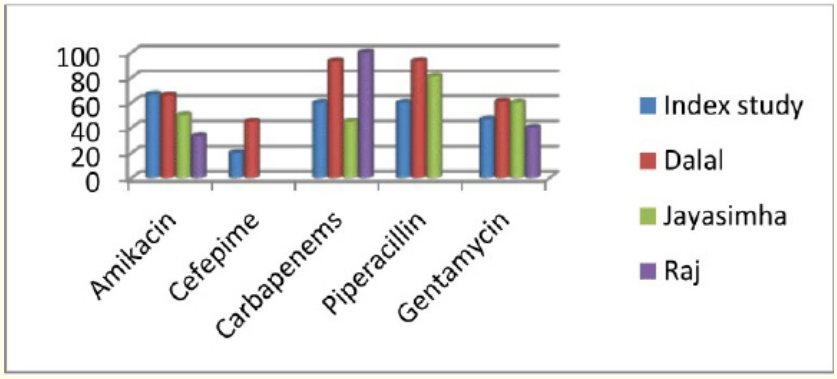

Figure 1c: *Shows the comparison of antibiotic sensitivity of Pseudomonas species among various Indian studies.*

Another important aspect was the need to use ciprofloxacin in *Klebsiella, E.coli* and *Staph. aureus* sepsis to avoid the use of reserved drugs like colistin, tigecycline and teicoplanin. Ciprofloxacin has no marketing authorization for use in neonates worldwide but it is still used for the treatment of neonatal life-threatening infections, mainly in developing countries and in Europe [27]. We used ciprofloxacin, since extensive literature review revealed that there were no serious adverse events, particularly joint toxicity, although evaluation was predominantly clinical and follow-up limited to few months after the end of treatment in those studies [27,28].

Discussion on mortality and morbidity

The study identifies preterm onset of labour, meningitis, PROM, and LBW to be significantly associated with neonatal mortality. The mortality rate in our study was 12.59% compared to 7% (Trotman) [26], 28% (Kalpana) [29], and 15.7% (Shobowale) [25] and 4% (Tewabe) [30]. The most novel part of this study was the evaluation of the effects of metabolic derangements (hypoglycemia, hypocalcemia and hyponatremia) and organ dysfunctions- haematological (thrombocytopenia and leucopenia), cardiovascular (shock), hepatic (coagu-

lopathy), renal (acute kidney injury) and respiratory (hypoxemia or hypercarbia necessitating mechanical ventilation) in septic neonates, which was not described before in any article from India. Prematurity, LBW, thrombocytopenia < 50,000, hypoglycemia, hypocalcemia, coagulopathy, renal failure, septic shock, meningitis and need for mechanical ventilation were significantly associated with poor neurodevelopmental outcome. This determination of the clinical risk profile sets the stage for the development of strategies directed at preventing poor outcome. The association of LBW and prematurity has also been demonstrated in other studies because the preterm infant is deficient in humoral and cellular immunity and are less likely to receive transplacental maternal antibodies as term infants. Trotman [26] described that there was no significant difference in outcome based on type of organism. However, Kalpana [29], Khatua [31] and Bhatia [32] described higher mortality in early onset and gram negative sepsis. Mitha [19] reported that neonates with combined EOS and LOS and with isolated LOS had a higher risk of cerebral palsy, but the association with isolated EOS was not significant after adjustment. In our study, the type of organism and the time of presentation (EOS/LOS) did not affect mortality or morbidity. Trotman [26] described higher mortality among male neonates and higher morbidity among female neonates perhaps because in his study the females were smaller and less mature than their male counterparts and therefore were at greater risk for complications from their sepsis. Mitha [19] reported that there was no difference in incidence of cerebral palsy on the basis of gender, and also antenatal corticosteroid therapy, similar to our study. He also described that EOS was more frequent in cases of PROM and LOS was significantly more frequent in neonates who were small for gestational age. The association of PROM with mortality was also described by Shobowale [25]. In his study 38.5% of neonates with PROM died compared to 16.79% in our study. Moreover, maternal illness and twin pregnancy was not associated with mortality in his study, similar to our results.

In developing countries, the reported incidence of neonatal meningitis is 0.8 - 6.1 per 1000 live births, with a mortality of 40 - 58% [33]. In our study, 74 (13.31%) infants had meningitis, mortality being 40.54%. 30 (68.18%) had developmental delay, compared to 40% in Mehkarkar's [34] study. The rate of mortality and developmental delay among infants with sepsis without meningitis was significantly lower 8.29% and 24.69% ($p < 0.0001$). A prospective study over 5 years with 1717 survivors of neonatal meningitis found that those with neonatal meningitis were 10 times more likely to have moderate or severe disability than children who never had meningitis, the rate of developmental delay being 23% [35]. Bhagat [36] described that infants with meningitis were at 8.2 and 6.5 times higher risk of mortality and developmental delay compared to infants with sepsis, but without meningitis. In his study, 17.6% patients with meningitis compared to 4.8% with sepsis, but no meningitis, expired ($p < 0.005$).

Limitations

Being retrospective, this study was limited by the fact that data collection was restricted to information previously recorded and this was incomplete for some of the variables under review. Lack of written hospital antibiotic policy during earlier phases is another drawback. However, this is the first study which correlates with clinical features, outcome and predictors of outcome for a large sample size.

Conclusion

The originality of this study resides in dealing with all aspects of culture proven neonatal sepsis- the causative organisms, their antibiograms, the outcome and the predictors of mortality and morbidity. This study adds data that *Klebsiella* species is still the predominant organism of neonatal sepsis in the Indian subcontinent, although significant rise in proportion of *Staphylococcus aureus* is occurring. The causative organism of neonatal sepsis as well as their antibiotic sensitivity pattern may change significantly within the same unit, mandating periodic surveillance of pathogens, and a need to strictly implement antibiotic policy to effectively curtail spread of these resistant organisms. Strategies aimed at decreasing the incidence of prematurity would eventually reduce morbidity and mortality in neonatal sepsis. The presence of organ dysfunction predicted poor neurodevelopmental outcome. Defining the clinical profile of the neonates at risk for poor outcome will promote early identification of neonates most in need of close monitoring and critical care, thus allowing for earlier initiation of aggressive therapy.

Funding

None.

Conflict of Interest

None.

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