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Original Article

Change in spectrum of microbial aetiology in relation to gestational age and birth weight and emergence of ESBL in tertiary neonatal intensive care units.

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ABSTRACT

Septicemia continues to be a major cause of neonatal mortality and morbidity worldwide. Aim: To study the incidence, microbial profile and emergence of extended spectrum beta lactamases mediated resistance (ESBL) in neonatal septicemia in relation to gestational age and birth weight. This is a prospective study of 1647 babies suspected of neonatal septicemia based on symptomatology and clinical diagnosis. Evaluated data included: age, sex, birth weight, type of isolated pathogen, and antibiotic sensitivity. Gram negative bacteria were the predominant organisms to be isolated. *Klebsiella spp* 232 (26.5%) was the predominant organism followed by *E. coli* 92(10.5%), and *Acinetobacter* 54(6.2%). Among Gram-positive bacteria, *Staphylococcus aureus* 176 (20.1%) was the predominant followed by coagulase negative Staphylococci 118(13.6%). Preterm babies were highly significantly more susceptible to infection than term babies (61.9% vs 40.4%; $P < 0.001$). *Klebsiella sps* is the predominant organism (36.7% & 23.8%) isolated among both preterm male and female babies followed by *Staphylococcus aureus* (14.4% & 14.7%). Among term babies *Staphylococcus aureus* (32.6%) was the predominant among the male babies followed by *E.coli* (23.5%) and *Staphylococcus aureus* (33.3%) was the predominant among female babies followed by CoNS (26.9%). Among the low birth weight babies, *Klebsiella sps* was isolated in 41.9% and in normal weight babies, *Staphylococcus aureus* (42.9%) was the predominant among the male babies and *Klebsiella sps* (32.3%) was the predominant among female babies. Multidrug resistant organisms were found to be most pathogenic. 32.8% of gram negative bacilli were ESBL producer *Klebsiella pneumoniae* was the predominant ESBL producer 47(43.9%) followed by *E.coli* 15 (14.0), and *Pseudomonas aeruginosa* 11(10.3%). AmpC production was seen in 18 (6.2%) of the isolates

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1. Introduction

The microbial profile of neonatal septicemia is constantly under change with advances in the early diagnosis and treatment of septicemia[1,2]. In the pre-antibiotic era, the most common organisms causing septicemia were Gram-positive cocci like *Streptococci pyogenes* and *Pneumococci*[3,4]. With the introduction of antimicrobial agents, Gram-negative organisms

like *E.coli*, *Pseudomonas* and *Klebsiella* continues to be a menace to the ill, fragile and debilitated newborns in the neonatal intensive care units, and the serotypes isolated are often resistant to multiple antibiotics[5-7] Thus these organisms continue to be a nightmare to neonatologists, microbiologists and hospital administrators[8]. Inadequate space, shortage of staff, high occupation rates, widespread use of antimicrobial agents and increased susceptibility of population, are responsible to early colonization and subsequent infection by virulent strains resulting in high morbidity and mortality[9-11]. The aim of the present work is to isolate and identify the organisms responsible for neonatal septicemia in relation to gestational age and birth weight in a tertiary neonatal intensive care units.

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2. Materials and Methods

A total of 1647 clinically diagnosed cases of septicemia were studied prospectively for over the period of 5 years. A detailed history of age, sex, birth weight, gestational age, and clinical symptoms of septicemia was recorded. Neonatal sepsis were suspected when any of the signs and systems or predisposing factors such as reduced activity, fever, refusal of feed, seizures, prolonged jaundice, birth asphyxia, umbilical sepsis, prematurity, abdominal distensions and history of premature rupture of membrane were noted in the newborns [12,13].

Two samples of blood were collected from each case using aseptic precautions. About 2 ml of blood was added immediately into 20 ml of brain heart infusion broth with 0.025% sodium polyethol sulphonate as anticoagulant [14]. The bottles were incubated for seven days and subcultures were done appropriately. The organisms were isolated and identified by standard microbiological techniques [15,16]. Antibiotic sensitivity pattern was evaluated by Kirby Bauer's disc diffusion methods [17]. Extended spectrum beta lactamases (ESBL) was detected by Double disk approximation test for screening and confirmed by NCCLS phenotypic confirmatory test [18, 19]. AmpC was detected by Amp C Disk Test. *E.coli* ATCC 25922 was used as control [20,21].

3. Results

Out of 1647 cases of neonatal septicemia, 877 (53.2%) were culture positive. In present study two blood samples were collected from all the neonates, of which in 78 cases the first sample was culture positive but the second sample did not show any growth. In 102 of 877 cases there was no organism isolated in the first sample, but the second sample was culture positive. Where as in 697 cases the same organism was isolated in the first and second blood samples (Table 1). This proves the importance of a second sample, which improves the isolation rate of the pathogen. However, certain authors have found results using a single blood culture to diagnose neonatal septicaemia (3,6,7). In such cases the incidence of culture positivity was less.

Table 1. Interpretation of Blood Culture

Total number of sample	Culture			770
	Positive in only 1 st sample	Positive only in 2 nd sample	Positive both 1 st & 2 nd sample	
1647	78	102	697	

Out of 877 positive cultures, were 474(54.0%) were gram-negative bacilli, followed by 307(35.0%) isolates of Gram-positive cocci and 96(10.9%) isolates of *Candida* spp.

Microbial profile of neonatal septicemia is depicted in table 2. Among the Gram-negative bacteria, *Klebsiella* spp 232 (26.5%) (*Klebsiella pneumoniae* 198, *Klebsiella oxytoxa* 34) was the predominant organism followed by *E.coli* 92(10.5%), *Acinetobacter* 54(6.2%) (*Acinetobacter baumannii* 38 and *Acinetobacter iwoffii* 03) *Pseudomonas aeruginosa* 42(4.8), *Enterobacter cloacae* 30(3.4%), *Citrobacter freundii* 21(2.3%) and *Alkaligenes faecalis* 03(0.3%) (Table 2)

Among Gram-positive bacteria, *Staphylococcus aureus* 176 (20.1%) was the predominant followed by coagulase negative *Staphylococci* 118(13.6%), (96 *Staphylococcus epidermidis* & 22 *Staphylococcus saprophyticus*), *Enterococci faecalis* 07(0.8%), *Streptococci viridans* 05(0.6%), and *Streptococci pneumoniae* 01(0.1%) (Table 2)

Table 2. Microbial Profile of Neonatal Septicemia

Organisms	No. of Isolates
<i>Klebsiella</i> spp	232(26.5%)
<i>Staphylococcus aureus</i>	176(20.1%)
Coagulase negative	118(13.6%)
<i>Staphylococci</i>	96(10.9%)
<i>Candida</i> spp/ <i>E.coli</i>	92(10.5%)
<i>Acinetobacter</i> spp	54(6.2%)
<i>Pseudomonas aeruginosa</i>	42(4.8%)
<i>Enterobacter cloacae</i>	30(3.4%)
<i>Citrobacter freundii</i>	21(2.3%)
<i>Enterococci faecalis</i>	07(0.8%)
<i>Streptococci viridians</i>	05(0.6%)
<i>Alkaligenes faecalis</i>	03(0.3%)
<i>Streptococci pneumoniae</i>	01(0.1%)
Total	877

Among *Candida* species (Plate-4.6 & 4.7), non-*Candida albicans* were predominant group followed by *Candida albicans*. Among non-*albicans*, *Candida tropicalis* 39(40.6%) was isolated in maximum number of cases followed by *Candida guilliermondii* 17 (17.7%), *Candida krusei* 14(14.5%), and *Candida parapsilosis* 04(4.1%). *Candida albicans* was isolated in 22(22.9%) cases.

a) Sex - More male babies were found to be culture positive. Among 963 males babies 532 (55.2%) were culture positive and out of 684 females babies 345 (50.4%) were culture positive. The difference in the culture positivity was statistically not significant (P>0.05).

b) Gestational age - Preterm babies were highly significantly more susceptible to infection than term babies (61.9% vs 40.4%; P<0.001). *Klebsiella* spp is the predominant organism (36.7% & 23.8%) isolated among both preterm male and female babies followed by *Staphylococcus aureus* (14.4% & 14.7%). Among term babies *Staphylococcus aureus* (32.6%) was the predominant among the male babies followed by *E.coli* (23.5%) and *Staphylococcus aureus* (33.3%) was the predominant among female babies followed by CoNS (26.9%) (table 3)

c) Birth weight - Culture positivity was highly significantly more in low birth weight (LBW) babies than in normal birth weight babies (61.3% vs 35.9%; P<0.001). *Klebsiella* spp was isolated in 41.9% among LBW babies followed by *Staphylococcus aureus* in 12.7%. Among normal weight babies, *Staphylococcus aureus* (42.9%) was the predominant among the male babies followed by CoNS (35.0%) and *Klebsiella* spp (32.3%) was the predominant among female babies followed by *Staphylococcus aureus* (19.4%) (table 4)

Table 3: Microbial Profile In Relation To Gestational Age In Culture Positive Cases

Bacterial isolates	Preterm		Full-term	
	MaleNo. (%)	FemaleNo. (%)	MaleNo. (%)	FemaleNo. (%)
<i>Klebsiella sps</i>	132(36.7)	60(23.8)	22(12.8)	18(19.4)
<i>Staphylococcus aureus</i>	52(14.4)	37(14.7)	56(32.6)	31(33.3)
CONS	41(11.4)	32(12.7)	20(11.6)	25(26.9)
<i>E.coli</i>	20(5.6)	13(5.2)	40(23.5)	19(20.4)
<i>Acinetobacter sps</i>	35(9.7)	15(5.9)	04(2.3)	-
<i>Pseudomonas aeruginosa</i>	12(3.3)	30(11.9)	-	-
<i>Enterobacter cloacae</i>	10(2.8)	20(7.9)	-	-
<i>Citrobacter freundii</i>	15(3.6)	06(2.4)	02(1.2)	-
<i>Enterococci feacalis</i>	03(0.8)	04(1.6)	-	-
<i>Streptococci viridians</i>	-	05(1.9)	-	-
<i>Alkaligenes feacalis</i>	03(0.8)	-	-	-
<i>Streptococci pneumoniae</i>	01(0.2)	-	-	-
<i>Candida sps</i>	38(10.6)	30(11.9)	28(16.3)	-
Total	360	252	172	93

Table 4: Microbial Profile In Relation To Birth Weight In Culture Positive Cases

Bacterial isolates	Birth weight <2500 gms		Birth weight ≥ 2500 gms	
	MaleNo. (%)	FemaleNo. (%)	MaleNo. (%)	FemaleNo. (%)
<i>Klebsiella sps</i>	165(41.9)	25(10.0)48	12(8.6)	30(32.3)
<i>Staphylococcus aureus</i>	50(12.7)	(19.1)43	60(42.9)	18(19.4)
CONS	14(3.6)	(17.1)39	49(35.0)	12(12.9)
<i>E.coli</i>	36(9.2)	(15.5)16	05(3.6)	12(12.9)
<i>Acinetobacter sps</i>	32(8.1)	(6.4)13	--	06(6.5)
<i>Pseudomonas aeruginosa</i>	25(6.4)	(5.2)13	--	04(4.3)
<i>Enterobacter cloacae</i>	17(4.3)	(5.2)13	-	-
<i>Citrobacter freundii</i>	10(2.0)	(5.2)02	-	-
<i>Enterococci feacalis</i>	05(1.3)	(0.8)05	-	-
<i>Streptococci viridians</i>	-	(2.0)	-	-
<i>Alkaligenes feacalis</i>	02(0.5)	-	-	01(1.1)
<i>Streptococci pneumoniae</i>	01(0.2)	-	-	-
<i>Candida sps</i>	38(9.7)	34(13.5)	14(10.0)	10(10.8)
Total	393	251	140	93

3.1. Antibiotic sensitivity pattern

Table 5 gives the antimicrobial susceptibility pattern of gram positive isolates while Table 6 gives the antimicrobial susceptibility pattern of gram negative isolates. All the 474 isolates of gram-negative were resistant to minimum of two antibiotics, hence all the isolates were considered multi-drug resistant. 76% of the isolates showed resistance or decreased susceptibility to at least one of the 3GC and 50% to all the 3GC. All the isolates were found sensitive to imipenem.

This information enables the clinician to administer appropriate antibiotic.

Table 5: Antimicrobial Susceptibility Pattern Of Gram Negative Isolates

Drugs		Klebsiella sps.	E.coli	Acinetobacter sps.	Pseudomonas aeruginosa	Enterobacter cloacae	Citrobacter freundii	Alkaligene feacalis
Ampicillin	S	218(94.0)	71(77.2)	42(77.8)	35(83.3)	28(93.3)	19(90.5)	00
	R	14(6.0)	21(22.8)	12(22.2)	07(16.7)	02(6.7)	2(9.5)	3(100)
Amikacin	S	114(49.1)	30(32.60)	16(29.6)	20(47.6)	11(36.7)	9(42.9)	00
	R	118(50.9)	62(67.4)	38(70.4)	22(52.4)	19(63.3)	12(57.1)	3(100)
Cefotaxime	S	152(65.5)	60(65.2)	41(75.9)	34(80.9)	24(80.0)	14(66.7)	00
	R	80(34.5)	32(34.8)	13(24.1)	08(19.1)	06(20.0)	7(33.3)	3(100)
Ceftazidime	S	142(61.2)	48(52.2)	28(51.9)	21(50.0)	21(70.0)	14(66.7)	00
	R	90(38.8)	44(47.8)	26(48.1)	21(50.0)	09(30.0)	7(33.3)	3(100)
Ceftriaxone	S	170(73.3)	70(76.1)	32(59.3)	24(57.1)	17(56.7)	11(52.3)	00
	R	62(26.7)	22(23.9)	22(40.7)	18(42.9)	13(43.3)	10(47.7)	3(100)
Ciprofloxacin	S	146(62.9)	50(54.3)	21(38.9)	32(76.2)	20(66.7)	13(61.9)	00
	R	86(37.1)	42(45.7)	33 (61.1)	10(23.8)	10(33.3)	8(38.1)	3(100)
Erythromycin	S	185(79.7)	59(64.1)	33(61.1)	34(80.9)	22(73.3)	14(66.7)	00
	R	47(20.3)	33(35.9)	21(38.9)	08(19.1)	08(26.7)	7(33.3)	3(100)
Gentamycin	S	193(83.2)	44(47.8)	39(72.2)	31(73.8)	17(56.7)	15(71.4)	00
	R	39(16.8)	48(52.2)	15(27.8)	11(26.2)	13(43.3)	6(28.6)	3(100)
Imipenem	S	0	0	0	0	0	0	00
	R	232(100)	92(100)	54(100)	42(100)	30(100)	21(100)	3(100)
Ofloxacin	S	124(53.4)	37(40.2)	12(22.2)	14(33.3)	17(56.7)	05(23.8)	00
	R	108(46.6)	55(59.8)	42(77.8)	28(66.7)	13(43.3)	16(76.2)	3(100)
Piperacillin	S	162(69.8)	45(48.9)	41(75.9)	15(35.7)	12(40.0)	11(52.3)	00
	R	70(30.2)	47(51.1)	13(24.1)	27(64.3)	18(60.0)	10(47.7)	3(100)

3.2.ESBL in neonatal septicemia

The isolates were chosen for detection of ESL was based on the MIC of ceftazidime and cefotaxime. As per the NCCLS screening criteria for ESBL those organisms which has MICs 2g/ml for ceftazidime or cefotaxime were screened for ESL detection and AmpC detection by 3-dimensional method.

Of the 474 gram-negative isolates, 326 isolates had MICs of 2g/ml for cefotaxime (as per the NCCLS screening criteria for ESL producing organisms). Out of 326 isolates, 152(65.9%) Klebsiella sps, had MICs of 2g/ml for cefotaxime (table 7). Similarly 60 (65.2%) E.coli, 41 (75.9%) Acinetobacter sps, 34(81.0%) Pseudomonas sps, 24 (80.0%) Enterobacter sps and 15(71.4%) Citrobacter sps had MICs of 2g/ml for cefotaxime. Table 8 illustrates number of organisms resistant to third generation

cephalosporins by disc diffusion method. 326 isolates were further taken for detection of ESBL by DDST method. The incidence of ESL production in neonatal septicemic cases is 107(32.8%) (table 9) Among gram-negative bacteria Klebsiella sps was the predominant ESBL producer (Klebsiella pneumoniae 47(43.9%) and Klebsiella oxytoca 04(3.7%)} followed by E.coli 15 (14.0), Pseudomonas aeruginosa 11(10.3%), Enterobacter cloacae 09(8.4%), Acinetobacter sps 06(5.6%) and Citrobacter sps 03(2.9%) (Table 6.10)

AmpC production was seen in 18 (6.2%) of the isolates by 3-dimensional test. Klebsiella sps (11) were the predominant AmpC producers followed by E.coli (04), Acinetobacter sps (03).

Table 6. Antimicrobial Susceptibility Pattern Of Gram Positive Isolates

Drugs		Staphylococcus aureus	CONS	Enterococci faecalis	Streptococcus viridians	Streptococcus pneumoniae
		(n=176)	(n=118)	(n=07)	(n=05)	(n=01)
Ampicillin	S	20(11.4)	48(40.7)	00	05(100)	01(100)
	R	156(88.6)	70(59.3)	07(100)	00	00
Amikacin	S	111(63.1)	79(67.0)	01(14.3)	05(100)	01(100)
	R	65(36.9)	39(33.1)	06(85.7)	00	00
Cefotaxime	S	108(61.4)	70(59.3)	01(14.3)	NT	NT
	R	66(37.5)	48(40.7)	06(85.7)	NT	NT
Ceftriaxone	S	94(53.4)	64(54.2)	03(42.9)	NT	NT
	R	82(46.6)	54(45.8)	04(57.1)	NT	NT
Clindamycin	S	118(67.0)	61(51.7)	04(57.1)	NT	NT
	R	58(33.0)	57(48.3)	03(42.3)	NT	NT
Ciprofloxacin	S	72 (41.0)	62(52.5)	00	NT	NT
	R	104(59.0)	56(47.5)	07(100)	NT	NT
Cephalexin	S	22(12.5)	52(44.1)	00	NT	NT
	R	154(87.5)	66(55.9)	07(100)	NT	NT
Erythromycin	S	60(34.1)	72(61.0)	00	05(100)	01(100)
	R	116(65.9)	46(39.0)	07(100)	00	00
Gentamycin	S	54(30.7)	68(57.6)	00	05(100)	01(100)
	R	122(69.3)	50(42.4)	07(100)	00	00
Norfloxacin	S	52(30.0)	47(39.8)	00	NT	NT
	R	124(70.0)	71(60.2)	07(100)	NT	NT
Ofloxacin	S	119(67.6)	92(78.0)	1(14.3)06	NT	NT
	R	57(32.4)	26(22.0)	(85.7)1	NT	NT
Oxacillin	S	102 (58.0)	25(75.4)	(14.3)06	NT	NT
	R	74(42.0)	25(21.2)	(85.7)	NT	NT
Penicillin	S	19(10.8)	42(35.6)	00	05(100)	01(100)
	R	157(89.2)	76(64.4)	07(100)	00	00

Table 7. Resistance Of Gram-negative Bacteria To Third Generation Cephalosporins

Organisms	Ceftriaxone	Ceftazidime	Cefotaxime
<i>Klebsiella</i> sps (232) *	170 (73.3)	142 (61.2)	152 (65.5)
<i>E.coli</i> (92)	70(76.1)	48(52.2)28	60(65.2)
<i>Acinetobacter</i> sps (54)	32 (59.3)	(51.9)21	41 (75.9)
<i>Pseudomonas aeruginosa</i> (42)	24(57.1)	(50.0)21	34 (80.9)
<i>Enterobacter cloacae</i> (30)	17(56.7)	(70.0)14	24 (81.0)
<i>Citrobacter freundii</i> (21)	11 (52.3)	(66.7)	14 (66.7)
<i>Alkaligenes faecalis</i> (03)	00	00	00

• Figures in the parenthesis indicate number of isolates

Table 8. Mic Range, Mic 50 And Mic 90 Of Cefotaxime Against Septicemic Pathogens

Septicemic pathogens	MIC range in g/ml	MIC 50 in g/ml	MIC 90 in g/ml
<i>Klebsiella</i> sps (232) *	0.003 – 512	16	256
<i>Staphylococcus aureus</i> (176)	0.003 – 128	0.12	64
<i>Coagulase negative staphylococci</i> (118)	0.006-128	0.25	32
<i>E.coli</i> (92)	0.006- 512	16	128
<i>Acinetobacter</i> sps (54)	0.012 – 256	32	128
<i>Pseudomonas aeruginosa</i> (42)	0.06 – 512	32	256
<i>Enterobacter cloacae</i> (30)	0.03 – 128	16	128
<i>Citrobacter freundii</i> (21)	0.06 – 128	16	32

* Figures in the parenthesis indicate number of isolates

Table 9. Distribution of ESBL Producing Strains Isolated From Neonatal Septicemia

Organisms	ESBL producers
<i>K. pneumoniae</i> (140)*	47 (43.9) ^a
<i>K. oxytoca</i> (12)	04(3.7)
<i>E. coli</i> (60)	15(14.0)
<i>Acinetobacter baumani</i> (38)	05(4.7)
<i>Acinetobacter lwofii</i> (03)	01(0.9)
<i>Pseudomonas aeruginosa</i> (34)	11(10.3)
<i>Enterobacter cloacae</i> (24)	09(8.4)
<i>Citrobacter</i> sps (15)	03(2.9)
Total (326)	107(32.8)

* Figures in the parenthesis indicate number of isolates

^a Figures in the parenthesis indicate percentage

4.Discussion

The epidemiology of neonatal septicemia in the developing and the industrialized countries shows some important differences in the pattern of etiological agents, which often changes over the years. The microbial profile of neonatal septicemia is constantly under change with advances in the early diagnosis and treatment of septicemia. In the pre-antibiotic era, the most common organisms causing septicemia were Gram-positive cocci like *Streptococci pyogenes* and *Pneumococci*. With the introduction of antimicrobial agents, Gram-negative organisms like *E.coli*, *Pseudomonas* and *Klebsiella* continues to be a menace to the ill, fragile and debilitated newborns in the neonatal intensive care units

Among 877 positive cultures isolated from 1647 neonates in the present study, 55.5% were Gram-negative bacilli, 33.5% were Gram-positive cocci and 10.9% were fungi. Study conducted by other workers reported the incidence of Gram-negative bacteria from 66.1 to 87.1% [8,9,22].

Klebsiella remains the most important pathogen in the nurseries in our country [5,10] followed by *Staphylococcus aureus*. One of the reasons expounded for the predominance of an organism in causing septicemia in the nursery is the selective pressure of antimicrobial agents, so that the resistant organisms tend to colonize and proliferates in the nurseries. This is also true with *Klebsiella* septicemia [11]. In the present study also *Klebsiella* with resistance to many antimicrobial agents was found to be the most frequent causative agent of septicemia.

Candida species are normally found on skin and mucous membrane of healthy individuals and therefore *Candidaemia* is generally an endogenous infection (Bhattacharya, 1983). *Candidaemia* is the most frequently encountered fungal infection especially in those babies with predisposing factors like prolonged antibiotic therapy, intravascular, catheterizations, endotracheal intubations, parenteral nutrition and artificial ventilation. In the present study, the factors that probably lead to *Candidaemia* were broad- spectrum antibiotic therapy, low birth weight and prematurity. Importance of *candida* as a pathogen in the nursery has been emphasized by many authors [23-28]. In our study 10.9% cultures yielded *Candida* species while we were evaluating for possible bacterial pathogens. With major advances in neonatal care, permitting very low birth weight neonates to survive, the incidence of all clinical forms of *Candida* infections is increasing [27, 28]. Parenteral nutrition was not a predisposing factor in our study, as the babies were breast-fed.

Maternal, perinatal and neonatal events put the baby at increased risk of infection. These factors play a major role in the incidence and the pattern of organisms in neonatal septicemia. The most significant factors in septicemia are low birth weight babies and prematurity. In India, the problem is magnified as a majority of admissions to the neonatal ICU comprises of septicemia. Some times septicemia may be the result of intensive life support measures used to survive pre-term babies or sick neonates. Pattern of organisms causing septicemia in low birth weight, normal birth weight, preterm babies and term babies differ from region to region and hospitals to hospitals.

The most common isolate in male preterm neonates is *Klebsiella* sps 132(36.7%) followed by *Staphylococcus aureus* 52(14.4%) and coagulase negative staphylococci 41(11.4%), where as in the full term neonates, *Staphylococcus aureus* 56(32.6%) followed by *E.coli* 40(23.5%) were the commonest isolate. Among the female neonates, *Klebsiella* sps 60(23.8%) and *Staphylococcus aureus* 37(14.7%) were the common isolates in preterm neonates followed by coagulase negative staphylococci 32(12.7%). In full term female neonates *Staphylococcus aureus* 31(33.3%) and coagulase negative staphylococci 25(26.9%) followed by *E.coli* 19(20.4%) were the commonest isolates.

In the study by Gupta preterm and low birth weight babies accounted for 85.5% of cases of *Klebsiella* septicemia [12] Maximum number of Coagulase negative Staphylococci was isolated from premature neonates in many intensive care units [29]. However, the causative organisms in premature babies were not different from full term babies [13]

Low birth weight neonates, both preterm as well as small for dates are much more handicapped as the result of deficiency of different components of immune system [14]. In the present study the incidence of neonatal septicemia was significantly high in low birth weight babies. However culture was positive only in 61.3% of low birth weight babies, which is comparable to 30.6 – 80.0% reported by others [15, 16, 30]. However, negative cultures could not rule out neonatal septicemia. In our study in low birth weight category, *Klebsiella* sps 165(41.9%) and *Staphylococcus aureus* 50(12.7%) were predominant in males and *Staphylococcus aureus* 48(19.1%) and Coagulase negative Staphylococci 43(17.1%) were predominant in female group.

In normal birth weight *Staphylococcus aureus* 60(42.9%) and Coagulase negative Staphylococci 49(35.0%) were predominant in male group and *Klebsiella* sps 30(32.3%), *Staphylococcus aureus* 18(19.1%) and *E.coli* 12(12.9%) were predominant in female normal birth weight babies. Das et al., (1999) found a higher incidence of neonatal infection in low birth weight babies (8.42%) with the common isolates being *Klebsiella*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. In normal birth weight of babies, coagulase negative Staphylococci was predominant [9, 31]

The emergence of antibiotic resistant organisms and their spread in the community has been a subject of concern in recent years. In the absence of specific surveillance, the prevalence of ESL in a country of region may be under recognized, as any routine susceptibility testing methods employed in clinical laboratories may not detect the production of ESBL. Approximately 30 % of the ESBL producers appeared falsely sensitive or moderately sensitive to cefotaxime\ ceftazidime in routine susceptibility testing. This is known that the MIC for ESLs producing organisms is higher than that for non-ESBL producers of the same species. However the MIC may not reach the breakpoint value for resistance and is thus reported as sensitive in routine disk diffusion susceptibility tests and also this method is performed under conditions that do not favor derepression of the enzymes because of failure of routine

4. Discussion

susceptibility test to detect resistance to the newer cephalosporins led to the institution of inappropriate therapy in some patients [32]. Similar problem have been reported with 3 GC in disk diffusion test. In most instances, the patients involved either relapsed or failed to respond to therapy. We found that the ESBL producing isolates were conferred with resistance or decreased susceptibility to various third generation cephalosporins. The DDST detected ESBL in 32.8% of the isolates. The specificity of DDST is well documented. Its sensitivity has been variable reported as 79% [33], 87% [34] and 93.3% [34]. In view of its simplicity, it may be undertaken in a routine diagnostic laboratory for detecting ESBL producing strains with due consideration to factors like precise placement of the discs, correct storage of the clavulanate containing discs and performance of appropriate control tests, which are critical to the sensitivity of the DDST.

Information on the prevalence of AmpC -lactamase producing strains in India is very limited, and no data's are available on the prevalence of AmpC production in neonatal group. In the present study 18(6.2%) isolates were resistant to cefoxitin were positive by 3 – dimensional test, negative for inducible lactamases by disc diffusional test, and sensitive to Imipenem.

5. Conclusion

Thus, the study clearly highlights the change in microbial profile and the rising level of drug resistance amongst the septicemic pathogens and hence the need to update the change in aetiology of neonatal septicemia and formulate newer drug policies. Good infection control practices, rational antibiotic policies, judicious use of interventions and implementation of standard of isolation precautions are of vital importance today. Unless there are strategies to optimize effective use of antibiotics, very few options will be left in future in the antibiotic armamentarium and it might herald an era of medical disaster with strains virtually untreatable with current spectrum of antimicrobials.

6. References

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