Original Research Paper

Volume-9 | Issue-6 | June-2019 | PRINT ISSN No. 2249 - 555X

Microbiology

ANTIBIOGRAM OF NEONATAL SEPTICEMIA IN A TERTIARY CARE HOSPITAL

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ABSTRACT Neonatal septicemia is a significant cause of mortality worldwide. As etiological agents and their antibiotics susceptibility pattern differs from place to place, the present study was performed.

Blood samples were collected from 566 clinically suspected cases of septicemia. The blood culture positivity rate was 36.22%. Gram negative organisms were found to be more common (70.24%) cause of sepsis than gram positives. *Klebsiella pneumoniae* was the predominant isolate showing maximum sensitivity to Imipenem and Amikacin. Among Enterobacteriaceae, ESBL production was seen in 61.62% of the isolates and AmpC production in 7.78%. Among non-fermenter group of organisms 33.33% isolates were MBL producers. In gram positive organisms the rate of MRSA was found to be 52.78%.

Neonatal septicemia is life threatening emergency, so early diagnosis and treatment with appropriate is necessary to avoid indiscriminate use of antibiotics

KEYWORDS : Septicemia, *Klebsiella pneumoniae*, Amikacin

INTRODUCTION

Neonatal septicemia is important cause of morbidity and mortality. It is responsible for approximately 25% of the neonatal deaths in the world[1] and mostly in developing countries.[2] Early diagnosis is a key to reduce morbidity and mortality of neonatal septicemia. The gold standard for diagnosis of septicemia is the isolation of bacterial agents from blood culture. Both Gram positive and Gram negative bacteria have been isolated from blood and predominance of one type over the other varies from place to place and even in the same place over time.[3] β-lactam antibiotics are commonly used among the neonatal septicemia cases. The emergence of resistance to these agents in the past decades has resulted in major clinical crisis. Resistance, especially to Cephalosporins due to production of extended spectrum β lactamases (ESBL) by Gram negative bacteria is on rise worldwide.[4,5] Carbapenem hydrolysing enzymes are most commonly seen in non-fermenter Gram negative bacilli that is Pseudomonas and Acinetobacter spp.[6] Hence surveillance is needed to identify common pathogens of neonatal septicemia as well as their antibiotic resistance pattern.

MATERIAL METHODS:

The observational study of 2 years was carried out from April 2014 to March 2016, in the department of Microbiology, Indira Gandhi government medical college, Nagpur. Clinically suspected cases of neonatal septicemia admitted in neonatal intensive care unit were included in the study. Two millilitres of blood was collected aseptically and preferably before administration of the antibiotics from 566 suspecetd neonates and inoculated into culture bottle containing 20 ml of trypticase soy broth. Blood samples were processed and bacterial isolates were identified by standard microbiological procedures.[7] Antimicrobial susceptibility of all bacterial isolates was done by Kirby-Bauer disk diffusion technique[8] as per CLSI 2014 guidelines.[9] For Testing of β-Lactamase production all isolates from Enterobacteriaceae family were tested for the production of extended spectrum β-lactamase (ESBL) and AmpC β-lactamase. For confirmation of ESBL production, Ceftazidime resistant (screening test +ve) strains were subjected to Disk Potentiation test.[9] For AmpC detection, Cefoxitin resistant (screening test +ve) strains were further tested by Cloxacillin combined disk diffusion test.[10] Metallo-β-lactamase (MBL) production was tested in Pseudomonas spp and Acinetobacter spp. Imipenem resistant strains were confirmed as MBL producers by Imipenem and Imipenem-EDTA disc potentiating test.[11] MRSA were detected by using a cefoxitin (30µg) disc.[9]

RESULTS

Out of total 566 blood samples subjected for culture, 205 were culture positive with the positivity rate of 36.22%. Male to female ratio was 1.66:1.

Table 1 show that Gram negative bacilli were the common cause of neonatal septicemia 144(70.24%). The most common pathogens were *Klebsiella pneumoniae* followed by *Pseudomonas aeruginosa*. Gram positive organisms were found in 61(29.76%) cases of which *S. aureus* were most common isolate.

Table 1: Distribution of organisms causing sepsis

SR NO	ORGANISMS ISOLATED	TOTAL (%)
Gram negative organisms		**144 (70.24)**
1	*Klebsiella pneumoniae*	54 (26.34)
2	*Pseudomonas aeruginosa*	27 (13.17)
3	*Escherichia coli*	23 (11.22)
4	*Acinetobacter baumannii*	14 (06.83)
5	Enterobacter spp	08 (03.90)
6	Citrobacter spp	08 (03.90)
7	*Klebsiella oxytoca*	06 (02.93)
8	*Acinetobacter lwoffii*	04 (01.95)
Gram positive organisms		**61 (29.76)**
9	*Staphylococcus aureus*	36 (17.56)
10	CONS	18 (08.78)
11	*Enterococcus faecalis*	05 (02.44)
12	*Streptococcus pneumoniae*	02 (00.98)
	Total	**205 (100)**

Table 2 shows antibiotic resistance pattern of the isolates from Enterobacteriaceae family. Maximum resistance was seen to Ampicillin 98(98.99%). High resistance was noted to Cephalosporines ranging from 51.52% to 76.77%. The most predominant isolate, Klebsiella spp 60 (*K.pneumoniae* 54+*K.oxytoca* 6) were least resistant to Imipenem 55(91.67%) and Amikacin 50(83.34%).

Table 2: Antibiotics resistance pattern of Enterobacteriaceae (n=99)

DRUGS	Klebsiella spp (n=60) (%)	E. coli (n=23) (%)	Citrobacter spp (n=08) (%)	Enterobacter spp (n=08) (%)	Total (n=99) (%)
Ampicillin	60 (100)	22 (95.65)	08 (100)	08 (100)	98 (98.99)
Amoxyclav	45 (75)	11 (47.83)	08 (100)	08 (100)	72 (72.73)
Ceftazidime	44 (73.33)	16 (69.57)	7 (87.5)	7 (87.5)	74 (74.75)
Cefotaxime	45 (75)	16 (69.57)	8 (100)	7 (87.5)	76 (76.77)
Cefepime	32 (53.33)	15 (65.22)	7 (87.5)	7 (87.5)	51 (51.52)
Cefoxitin	22 (36.67)	8 (34.78)	08 (100)	08 (100)	46 (46.46)
Pipera-Tazo	18 (30.00)	8 (34.78)	4 (50.00)	5 (62.5)	35 (35.35)
Aztreonam	38 (63.33)	16 (69.57)	6 (75)	4 (50.0)	64 (64.65)
Imipenem	5 (08.33)	3 (13.04)	1 (12.50)	1 (12.5)	10 (10.10)
Gentamicin	15 (25.00)	7 (30.43)	4 (50.00)	4 (50.0)	30 (30.30)
Amikacin	10 (16.66)	5 (21.73)	2 (25.00)	1 (12.5)	18 (18.18)
Ciprofloxacin	25 (41.67)	8 (34.78)	2 (25.00)	2 (25.0)	37 (37.37)

Out of total 99 isolates, 61(61.62%) were confirmed as ESBL producers. Maximum ESBL producers were Klebsiella spp (70%) and *Escherichia coli* (69.56%). Among Cefoxitin resistant isolates, 07(7.78%) were confirmed as AmpC producers. There was co-production of ESBL and AmpC in 3(3.03%) isolates. (Table3)

Table 3: β-lactamase production among Enterobacteriaceae (n=99)

Organisms	ESBL		Amp C		Co-production of ESBL+AmpC (%)
	Screening test positive (%)	confirmatory test positive(%)	Screening test positive(%)	confirmatory test positive(%)	
Klebsiella spp (n=60)	44(73.33)	42(70.00)	22(36.67)	04(6.67)	02 (3.33)
Escherichia coli (n=23)	18 (78.26)	16(69.56)	8 (34.78)	01(4.35)	01(4.35)
Enterobacter spp (n=08)	7 (87.5)	01 (12.50)	08 (100)	01 (12.50)	-
Citrobacter spp(n=08)	7 (87.5)	02 (25.00)	08 (100)	01 (12.50)	-
TOTAL *(n=99)*	76 (76.77)	**61 (61.62)**	46 (46.47)	**07(7.78)**	**03(3.03)**

Antibiogram of *P.aeruginosa* and Acinetobacter spp. shows that more resistance was noted to Gentamicin, Ceftazidime and Cefotaxime. (Table 4)

Table 4: Antibiotic resistant pattern of *P.aeruginosa* and Acinetobacter spp.

DRUGS	*P. aeruginosa* n=27(%)	*A. baumanii* n=14(%)	*A. lwoffii* n=4(%)
Ceftazidime	13 (48.15)	9 (64.29)	4 (100)
Cefotaxime	--	10 (71.43)	4 (100)
Cefepime	12 (44.44)	7 (50.0)	2 (50)
Pipera-Tazo	11 (40.74)	6 (42.86)	2 (50)
Aztreonam	18 (66.67)	9 (64.29)	2 (50)
Imipenem	10 (37.04)	4 (28.57)	2 (50)
Colistin	0(0)	--	--
Gentamicin	14 (51.85)	12 (85.71)	3 (75)
Amikacin	5 (18.52)	5 (35.71)	1 (25)
Ciprofloxacin	16 (59.26)	9 (64.29)	2 (50)

All the 10 Imipenem resistant Pseudomonas isolates were MBL producers, while 5 of the 6 Acinetobacter spp isolates were MBL producers. This indicates the mechanism of resistance in that strain may be other than the production of metalo-beta-lactamases. (Table 5)

Table 5: Metallo β- lactamases (MBL) detection

Organisms	MBL	
	Screening test positive(%)	confirmatory test positive(%)
***P. aeruginosa* (n= 27)**	10 (37.04)	10 (37.04)
***Acinetobacter spp* (n= 18)**	06 (33.33)	5(27.78)
TOTAL *(n=45)*	16(35.56)	**15(33.33)**

Out of 36 isolates of *S.aureus*, 6(16.67%) were resistant to Amikacin while Methicillin resistance was seen in 19(52.78%) of the *S.aureus.* (Table 6)

Table 6: Antibiotic resistance pattern of Gram positive organisms (n= 61)

DRUGS	*S. aureus* (n=36) (%)	CONS (n=18) (%)	*Enterococcus faecalis (n=05)(%)*	*Streptococcus pneumonia (n=02)(%)*
Penicillin	34 (94.44)	16 (88.89)	3 (60)	--
Ampicillin	--	-	3 (60)	--
Cefoxitin	19 (52.78)	10 (55.56)	-	--
Gentamicin	12 (33.33)	8 (44.44)	-	--
Amikacin	6 (16.67)	4 (22.22)	-	--
High L Gen	-	-	0	--
Ciprofloxacin	16 (44.44)	8 (44.44)	2 (40)	--
Linezolid	0	0	0	0

DISCUSSION:

The detection of microorganisms in a patient's blood has great diagnostic and prognostic significance, particularly, in neonates with suspected sepsis. In the present study, blood culture positivity in neonatal septicemia cases was 36.22%. Our findings were similar to the prevalence rate of 32% reported by Gandhi S *et al*[12] from India. In the present study, male cases outnumbered the female cases with ratio of 1.66:1 and Gram-negative organisms predominated being responsible for 70.24% of cases of septicemia. Similar findings were made by Muley VA *et al*.[13] The probable reasons being, newborns most probably acquire these Gram-negative rods from the vaginal and faecal flora of the mother and the environment where the delivery occurs.[14] In present study, the most frequently isolated organism was *Klebsiella pneumoniae* 54(26.34%) followed by *S.aureus* 36 (17.56%). *Our findings were similar to Muley VA et al.*[13]

Antibiotic resistance is today a global problem. In the present study also greater prevalence of resistance has been noted to commonly used antibiotics. High degree of resistance to the penicillin and cephalosporins was the cause of concern. Maximum sensitivity was seen to Imipenem and Amikacin in Gram negative bacilli; and to Linezolid and Amikacin in Gram positive cocci. The wide availability of over the counter antibiotics and the inappropriate use of broad spectrum antibiotics in the community may explain this situation. Enterobacteriaceae producing both ESBLs and AmpC β- lactamases have been increasingly reported worldwide.[15] The high rate of ESBL and Amp C producers in our setting is alarming. Carbapenems are the reserved drugs and the choice for the therapeutic management for multiple antibiotic resistant isolates like ESBL and AmpC producers. But the spread of carbapenem-resistant bacteria especially MBL producers have caused grave concern due to the limited choice of antibiotics for treating infections. Our results were comparable with Chelliah A et al[16].

MRSA is currently recognized as a major problem in hospitals and especially in intensive care units. Globally, 40-60% of *Staphylococcus aureus* strains are resistant to Methicillin.[13] In the present study, rate of MRSA was 52.78% and all of them were sensitive to Linezolid.

CONCLUSION

There should be institutional based 'Sepsis management protocol' to guide choices of antibiotics for management which will halt further increase in antibiotic resistance and will improve prognosis.

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