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Bacteriological profile and antimicrobial susceptibility pattern of aerobic bacteria isolated from blood stream of septicemic newborn admitted in NICU in tertiary care hospital of Bikaner

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Abstract
Aims & Objective: To determine causative aerobic bacteria and their antibiotic susceptibility pattern in cases of neonatal sepsis admitted in NICU of PBM & associated group of hospitals, Sardar Patel Medical College, Bikaner.
Materials & Method: Out of 9975 live born babies delivered at women hospital of PBM and associated group of hospitals, Blood culture samples were collected from 330 clinically suspected cases of neonatal sepsis, admitted in NICU during the period from July 2016 to June 2017. The blood samples were inoculated into Brain Heart Infusion Broth for aerobic culture and subcultures were performed on Blood agar, Mackonkey agar. The isolated microorganisms were identified as per standard protocol and antibiotic sensitivity pattern determined by using Kirby Bauer disc diffusion method on MHA as per CLSI guidelines.
Results: Out of 9975 cases incidence rate of neonatal sepsis was 1.87%. Neonatal sepsis observed more common in male 110(58.82%) than female 77(41.17%).Early onset sepsis (EOS) was found in 104(55.61%) cases whereas the rest 83(44.38%) cases were of Late onset sepsis (LOS). Staphylococcus aureus 70(37.43%) and Klebsiella pneumonia 19(10.16%) were the most common gram positive and Gram negative bacteria causing neonatal sepsis respectively. Gram positive bacteria were highly sensitive to vancomycin and shows poor sensitivity against azithromycin, cefpodoxime and cefotaxime. Gram negative bacteria were highly sensitive to meropenem & poor sensitive against ampicillin, cefazolin, cefaperazone and amoxyclav. Out of the 70 isolates of COPS 50% were MRSA. ESBL production was seen in 33.33% of E. coli and 26.31% of Klebsiella pneumoniae.
Conclusion: Empirical treatment of neonatal sepsis wit ampicillin, azithromycin, cefpodoxime, cefotaxime, cefazolin, cefoperazone, and amoxiclav in NICU of PBM Hospital Bikaner must be reconsidered. Determination of sensitivity pattern of isolates to formulate policy to use antibiotics in NICU emphasized.

Keywords: neonatal sepsis, BHI, MRSA, ESBL, blood agar, CLSI

1. Introduction
Neonatal sepsis refers to generalised bacterial infection documented by a positive blood culture in the first month of life [1]. Neonatal sepsis can be classified into two major categories depending up on the onset of symptoms: Early onset sepsis (EOS) presents within the first 72 hours of life and late onset sepsis (LOS) usually presents after 72 hours of age [2]. Infants with EOS usually present with respiratory distress and pneumonia. The source of infection is generally the maternal genital tract, organism present in labour room and operation theatres [2]. The source of infection in LOS is either nosocomial (hospital-acquired) or community-acquired and neonates usually present with septicaemia, pneumonia or meningitis [3, 4].
Neonatal Sepsis is an important cause of morbidity and mortality among neonates in India, with estimated incidence of approximately 4% in intramural live births [5]. Prior to antibiotic era, the mortality from septicaemia was 90% but it declining to 24%-58% after antibiotic came in use [6]. Sepsis related mortality is largely preventable by applying prophylactic measures of sepsis itself, timely recognition,

rational antimicrobial therapy and aggressive supportive care.

Incidence of septicaemia varies from one institute to another and even year to year in same institute. Growth of pathogenic microorganisms on blood culture is the most specific method for diagnosis of neonatal septicemia. Organisms responsible for neonatal septicemia have shown significant variation over the years. In addition, bacteria most frequently responsible for neonatal sepsis vary between different countries.

In developing countries Staphylococcus aureus and Klebsiella are dominants but contrasts with developed countries where Group B streptococci (GBS) and coagulase negative staphylococcus dominants in early and late onset sepsis respectively.

The susceptibility of the isolates to different antibiotics varies and also there is increasing concern of isolation of highly antibiotic resistant organisms. The purpose of this study was isolation and identification of aerobic bacteria causing neonatal sepsis in our NICU with their antibiotic sensitivity pattern.

2. Materials & Method

This prospective study was conducted in Department of Microbiology in collaboration with paediatrics department, Sardar Patel Medical College, Bikaner from July 2016 to June 2017. Studied neonates were divided into two groups according to timing of clinical signs as early-onset (clinical signs of sepsis from birth to 72 hours) or late onset (clinical signs of sepsis after 72 hours to 28 days old). Growth of pathogenic microorganisms on blood culture is the most specific method for diagnosis of neonatal septicemia, so blood culture used as the criteria of Neonatal sepsis. The growth of bacteria is evident in most blood cultures within 48 hours after incubation. Out of 9975 live born babies Delivered at women hospital of PBM and associated group of hospital during the period of one year, blood culture samples were collected from 330 clinically suspected cases of neonatal sepsis admitted in NICU of tertiary care hospital, Bikaner.

Sample collection

After obtaining permission from parents, blood was collected for blood culture from a peripheral vein by a 21 gauge needle with 5ml syringe after cleaning the skin with iodine and alcohol. Sample of blood was collected before second dose of antibiotic in all septicemic neonates. Cap of blood culture bottle was cleaned with surgical spirit and the needle of sterile syringe was plunged through the cap and 2ml blood was added to brain heart infusion broth (the amount of blood was collected 1/10 of Brain Heart Infusion Broth).

Sample processing

The samples were processed for the identification of organism by the conventional method. After inoculation, the blood sample was incubated at 37 ºC under aerobic condition in the incubator for 7 days. Three subculture were made, first after 24 hours, then at 72 hours and on the 7th day on Blood agar and MacConkey agar. Any growth was identified by colonial characteristics, Gram's stain and standard biochemical tests. Culture which did not yield any growth following three subcultures was reported negative at the end of 07 days. Antibiotic sensitivity testing was performed by Modified Kirby-Bauer disc diffusion method as per CLSI recommendations.

3. Results

Out of the 330 samples, collected from clinically suspected cases of Neonatal sepsis, blood culture was positive in 187(56.66%) cases (Table2). Among these, 134(71.65%) had sepsis with gram positive and 53(28.35%) with gram negative bacteria. Overall incidence of neonatal sepsis was 1.87% (Table 1). Incidence of blood culture positivity was more in male 110(58.82%) than female 77(41.17%) (Table1). EOS was found in 104(55.61%) cases whereas the rest 83(44.38%) cases were of LOS (Table2 & Figure: 1). Staphylococcus aureus 70(37.43%) and Klebsiella pneumoniae 19(10.16%) were the most common gram positive and gram negative bacteria causing neonatal sepsis in our NICU respectively (Table3 & Figure:2).

Gram positive bacteria were highly sensitive to vancomycin and shows poor activity against azithromycin, cefpodoxime, and cefotaxime (Table 4, Fig 3). Gram negative bacteria were highly sensitive to meropenem and shows poor activity against ampicillin, cefazoline, cefoperazone, amoxyclav (Table: 5 & Figure: 4). Pseudomonas aeruginosa exhibited poor activity against Ciprofloxacin 3(50%) and were sensitive to meropenem, Colistin, polymyxin-B (Table 5 & Figure: 4). Out of the 70 isolates of COPS 50% were MRSA. ESBL production was seen in 33.33% of E.coli and 26.31% of Klebsiella pneumoniae.

List of Tables

Table 1: Incidence of neonatal sepsis in male and female

Sex	Total no. of live births			Cases of Neonatal sepsis (By positive blood culture)		
	Male	Female	Total	Male	Female	Total
	5155 (51.68%)	4820 (48.32%)	9975	110(58.82%)	77(41.18%)	187 (1.87%)

Table 2: Distribution of cases of Neonatal sepsis according to age of onset

Age of onset	Culture Positive	Culture Negative	Total
Eons	104 (55.61%)	75 (52.45%)	179 (54.25%)
Lons	83 (44.39%)	68 (47.55%)	151 (45.75%)
Total	187 (56.66%)	143 (43.33%)	330

Table 3: Bacterial isolates in blood cultures

Microorganisms	Eons n (%)	Lons n (%)	Total n (%)
Staphylococcus aureus	49(47.11%)	21(25.30%)	70(37.43%)
CONS	13(12.50%)	30(36.14%)	43(22.99%)
Klebsiella pneumoniae	14(13.46%)	5(6.02%)	19(10.16%)
Acinetobacter spp.	6(5.76%)	12(14.45%)	18(9.62%)
Enterococcus	12(11.53%)	4(4.81%)	16(8.55%)
E. coli	5(4.80%)	4(4.81%)	9(4.81%)
Pseudomonas aeruginosa	2(1.92%)	4(4.81%)	6(3.20%)
Group-D streptococcus	3(2.88%)	2(2.40%)	5(2.67%)
Citrobacter spp.	0	1(1.20%)	1(0.53%)
Total	104(55.61%)	83(44.39%)	187

Table 4: Antibiotic sensitivity pattern of the Gram positive bacterial isolates

Antibiotics	COPS (70)	CONS (43)	Enterococcus (16)	Group D-streptococcus (5)	Total (134)
Vancomycin	70(100%)	43(100%)	15(93.75%)	5(100%)	133(99.25%)
Oxacillin	35(50%)	22(51.16%)	4(25%)	3(60%)	64(47.76%)
Cephotaxime	30(42.85%)	21(48.83%)	5(31.25%)	4(80%)	60(44.77%)
Ceftriaxone	31(44.28%)	28(65.11%)	8(50%)	4(80%)	71(52.29%)
Azithromycin	27(38.57%)	16(37.20%)	5(31.25%)	4(80%)	52(38.80%)
Ciprofloxacin	42(60%)	39(90.69%)	11(68.75%)	3(60%)	95(70.89%)
Cefpodoxime	28(40%)	22(51.16%)	5(31.25%)	3(60%)	58(43.28%)
Clindamycin	48(68.57%)	29(67.44%)	6(37.5%)	4(80%)	87(64.92%)
Gentamycin	43(61.42%)	35(81.39%)	8(50%)	4(80%)	90(67.16%)

Table 5: Antibiotic sensitivity pattern of the Gram negative bacterial isolates

Antibiotics	Klebsiella pneumonia (19)	E. coli (9)	Acinetobacter (18)	Citrobacter (1)	Pseudomonas aeruginosa (6)	Total 53 (%)
Ampicillin	2(10.52%)	0(0%)	3(16.66%)	0(0%)	-	5(10.63%)
Amoxyclav	3(15.78%)	2(22.22%)	4(22.22%)	0 (0%)	-	9(19.14%)
Ceftriaxone	12(63.15%)	6(66.66%)	6(33.33%)	0(0%)	-	24(51.06%)
Meropenem	19(100%)	9(100%)	18(100%)	1(100%)	6(100%)	53(100%)
Piperacillin+Tazobactam	13(68.42%)	6(66.66%)	12(66.66%)	0(0%)	5(83.33%)	36(67.92%)
Cefoperazone	3(15.78%)	2(22.22%)	4(22.22%)	0(0%)	-	9(19.14%)
Cefazoline	3(15.78%)	3(33.33%)	3(16.66%)	0(0%)	-	9(19.14%)
Gentamycin	7(36.84%)	7(77.77%)	10(55.55%)	0(0%)	5(83.33%)	29(54.71%)
Ciprofloxacin	12(63.15%)	6(66.66%)	10(55.55%)	0(0%)	3(50%)	31(58.49%)
Colistin	-	-	-	-	6(100%)	6(100%)
Cefepime	-	-	-	-	4(66.66%)	4(66.66%)
Aztreonam	-	-	-	-	4(66.66%)	4(66.66%)
Ceftazidime	-	-	-	-	4(66.66%)	4(66.66%)
Polymyxin-B	-	-	-	-	6(100%)	6(100%)

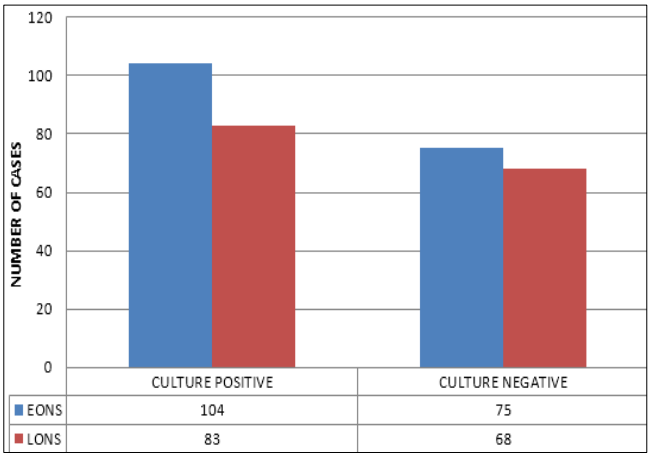

Fig 1: Distribution of cases according to age of onset

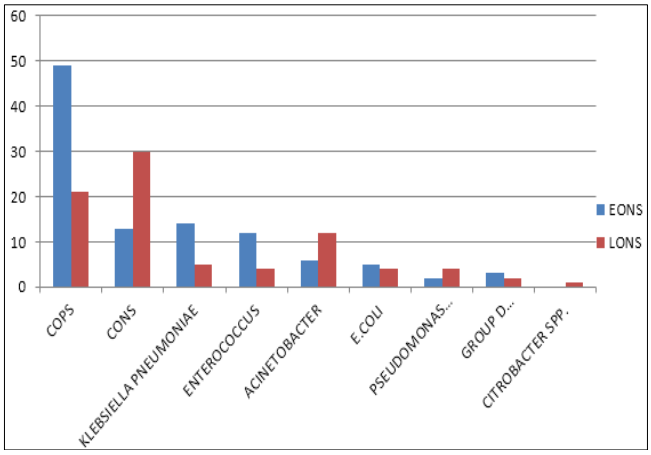

Fig 2: Bacterial isolates in blood culture

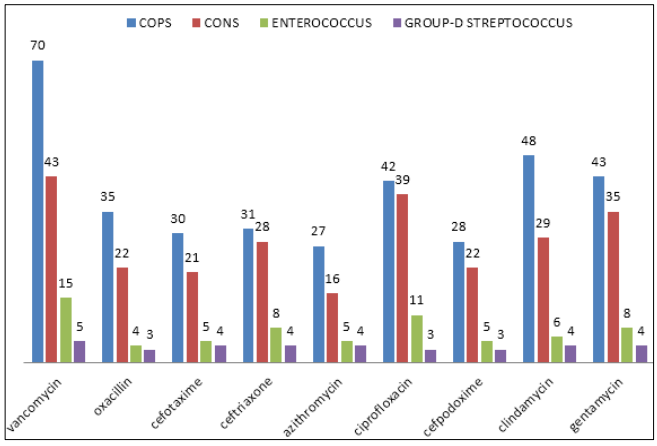

Fig 3: Antibiotic sensitivity pattern of Gram positive bacterial isolate**s**

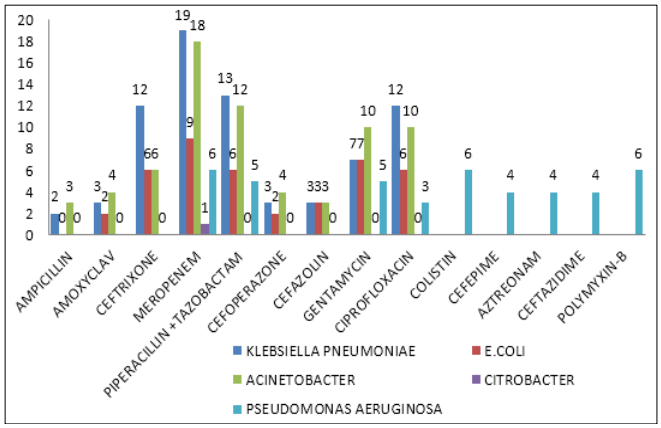

Fig 4: Antibiotic sensitivity pattern of the Gram negative bacterial isolates

4. Discussion

In the present study criteria for diagnosis of neonatal sepsis was blood culture positivity alone. Overall incidence of neonatal septicemia among live born babies was found to be 1.87% of live birth (Table 1). Incidence of septicemia varies from one institute to another and even year to year in same institute. In comparison to present study, Choudhary *et al.* [7] reported a much higher incidence of neonatal septicemia i. e. 11.2% live birth. The higher incidence of neonatal sepsis was due to the fact that their diagnosis of neonatal sepsis was based on clinical features alone.

As similar to our study G. karthikeyan and premkumar[8] reported much lower (1.96%) incidence of neonatal sepsis. This may be due to the fact that the criterion for diagnosis of septicemia was blood culture positivity alone. Neonatal septicemia was found to be more common in males in present study being 58.82% as compared to female 41.18%. (Table-1) The factors regulating the synthesis of gamma globulin are probably situated on X chromosomes in the male infants thus confers less immunological protection compare to female counterpart [9]. Maimuna *et al.* [10] also reported higher incidence of sepsis in males (61.2%) as compare to female neonates (38.8%).

In this study, incidence of early onset sepsis was 55.61% while late onset (44.39%) (Table 2). Maimuna Mustafa *et al.* [10] reported development of early onset sepsis was 58% and late onset was 42% which was similar to our observation. Our findings were consistent with that of Choudhary *et al.* [11] and Anil *et al.* [12] while Bhide *et al.* [13] reported early onset in 80% and late onset in 20%. Blood culture was positive in 56.66% of septicemic neonates in present study (Table 2), which is comparable to G karthikeyan and k. premkumar [8] who observed 51.34 blood culture positive cases. Staphylococcus aureus 70(37.43%) and Klebsiella pneumoniae 19(10.16%) were the most common gram positive and gram negative bacteria causing neonatal sepsis in our NICU respectively (Table 3, Fig 2). Similar to our study Gayawali *et al.* [14] observed that Staphylococcus aureus remained the predominant isolate followed by Klebsiella spp. About 35(50%) of Staphylococcus aureus were found to be MRSA which is similar to study conducted by Sneha S Hegadi and Kalpana [15] in 2012 year which were 21(52.5%) of total COPS.

Some studies shows different results from our study, as Balaka *et al.* [16] observed that Enterobacteriaceae (54%) and S. aureus (28%) were the most frequent microorganisms and particularly during the first week of life. The pattern of isolates of our study agrees with that of other developing countries where Staphylococcus aureus and klebsiella dominates but contrasts with developed countries where Group B streptococci (GBS) and coagulase negative staph. dominate in early and late onset sepsis respectively. This may be attributable to low prevalence of GBS colonization in pregnant mother in our country or, possibly, to the presence of strain with low virulence.According to our study Gram negative bacteria were highly resistant to ampicillin, amoxyclav, cefazoline, cefoperazone, and highly sensitive to piperacillin + tazobactam (Table 5 & figure: 4). All gram negative bacteria were sensitive to meropenem. Ceftriaxone shown good activity against E.coli and Group–D streptococcus. Piperacillin + Tazobactam shown better results against Klebsiella and Pseudomonas.

Piperacillin had advantage over ampicillin. As all E. coli isolates were resistant to ampicillin. Citrobactor was sensitive to meropenem. (Table 5). Pseudomonas aeruginosa exhibited poor activity against Ciprofloxacin 3(50%) as compared to meropenem, colistin, polymyxin-B which sensitive against all pseudomonas isolates. Vancomycin had good activity against gram positive organisms (enterococcus, CONS, MRSA, Group-D streptococcus) (table 6). Most of the gram positive isolates exhibited higher resistance to azithromycin and cephalosporins (Table 4, fig 3). Some studies show different results from present study. Desai *et al.* [17] concluded that Majority of organisms isolated were resistant to commonly used antibiotics. Maximum sensitivity was seen by Cefoperazone (97%) & Piperacillin/tazobactum (98%) for Gram negative organisms. The results obtained by present study were comparable to other studies conducted in our country in respect to the higher incidence, and their antibiotic susceptibility pattern and causative organisms of sepsis.

5. Conclusion

From this study we noticed that gram positive organisms like Staphylococcus aureus, Coagulase negative staphylococcus and gram negative klebsiella pneumoniae were predominant pathogens found in nearly 70.58% cases which is similar to other studies in our country. Present study, showed alarming results of antibiotic sensitivity patterns of antibiotics which are routinely used like azithromycin, ampicillin, cefaperazone, cefotaxime, cefazoline and amoxyclav. Ceftriaxone showed poor activity against most of the organisms. Empirical treatment of neonatal sepsis with ampicillin, azithromycin cefpodoxime,

Cefotaxime, cefazolin, cefapera-zone, and amoxyclav in NICU of PBM Hospital Bikaner must be reconsidered.

In fact, the sensitivity pattern varies in different parts of the same country as well as at various times in the same hospital, due to frequent emergence of resistant strains. Due to small sample size and short duration of study, we recommend additional prospective long term regular studies of antibiotic sensitivity of pathogens in order to formulate rational antibiotic policies. So that it contributes towards a more rational and appropriate use of antibiotics thus minimizing the emergences of multidrug resistant bacteria in neonatal unit. In our set up, we should analyze our quarterly culture reports and sensitivity pattern to formulate policy to use antibiotics in next three months. Using antibiotic in rotation may be effective in reducing resistance. In the last, but not the least, prevention of infection should not be neglected. Improved hand washing, use of disposables, early introduction of enteral feeds, preferably breast milk to babies, cannulas to be removed timely to reduce the risk of sepsis. In this scenario, strategies to reduce sepsis and its medical, social and economical toll need to address urgently.

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