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Bacteriological Profile and their Antibiotic Susceptibility Pattern in Neonatal Bacteremia

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Neonatal Bacteremia is the presence of live bacteria circulating in the blood of neonates. The bloodstream infections are important cause of neonatal mortality and morbidity. This study was done to know about the bacterial agents causing neonatal Bacteremia and their antibiotic sensitivity pattern. Blood cultures were done on clinically suspected bloodstream infections in neonates for a period of 18 months. 200 neonatal blood samples were cultured by conventional method. Bacterial isolates were identified and their antibiotic susceptibility testing were done. Out of 200 blood samples 88 samples(44%) showed positive blood culture. Blood culture was positive in 51 males(57.95%) and 37 females(42.1%).Among the positive blood culture samples, 66 bacterial isolates were Gram positive(75%) and 22 isolates were Gram negative(25%). *Staphylococcus aureus*(54.5%) was the most common bacteria isolated followed by coagulase negative *Staphylococcus* species(20.5%).Among the Gram negative organisms, *E.coli* was isolated in 13.6% of positive blood culture samples followed by *Klebsiella pneumoniae*(9.1%) and *Pseudomonas* sp.(2.3%). Gram positive organisms were highly resistant to oxacillin(88.8-89.61%).They were sensitive to Linezolid(93.75-94.5%),Teicoplanin(83.4-87.5%), Vancomycin(81.25-83.33%) and Amikacin(81.25-83.4%). Gram negative organisms were mostly resistant to third generation cephalosporins and gentamycin. They were mostly sensitive to amikacin and piperacillin. Prompt treatment of positive blood culture neonates with appropriate antibiotics can prevent the complications and can reduce the mortality.

Keywords: Bacteremia, coagulase negative *Staphylococcus*, blood culture, Antibiotic susceptibility testing, multidrug resistance.

First 28 days of life after birth is called the neonatal period and bloodstream infections occurring in this period can lead to significant morbidity and mortality in neonates. Bacteremia in neonates is associated with fever, refusal of feeds, tachycardia, tachypnoea, convulsions, bulging fontanelle , hypoglycemia and reduced movements. Bacteremia occurring in early neonatal period may be due to maternal factors like early rupture of membranes or intrapartum infections. Bacteremia occurring in late neonatal period may be community acquired or hospital acquired infections[1]. Neonatal bacteremia is commonly caused by Group B *Streptococci, E.coli, Staphylococcus aureus,* CONS, *Listeria monocytogenes*, *Klebsiella* sp.and other Gram negative bacteria. Bacteremia in late neonatal period is commonly associated with *Staphylococcus* sp. and Gram negative bacteria like *E.coli* and *Klebsiella* sp. These organisms are prevalent in the hospital environment and contact with colonized persons of *Staphylococcus* sp. may be the source of infection. Neonates with assisted ventilation may be of risk with *Pseudomonas* infections[2]. MRSA and ESBL producers and

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multidrug resistant Gram negative organisms are to be considered in the treatment of neonatal bacteremia .This study was conducted to know about the bacteriological agents causing neonatal bacteremia and their susceptibility pattern. This will help in the treatment of neonatal bloodstream infections which is one of the leading cause of neonatal morbidity and mortality[3].

MATERIALS AND METHODS

Study period

18 months from Jan 2016 to Jun 2017.

Type of study

cross sectional prospective study

Sample size

200 neonatal blood culture samples

Inclusion criteria

Neonates with features suggestive of bloodstream infections associated with systemic inflammatory response with no localized focus of infection.

Exclusion criteria

Neonates who had received prior antibiotics and birth weight less than 1000 gms.

Neonatal risk factors

Low birth weight, prematurity, meconium aspiration, birth asphyxia, indwelling intravenous canula for more than 10 days and continuous positive airway pressure.

Maternal risk factors

Early rupture of membranes, genitourinary infections, recurrent abortions and amniocentesis.

Procedure

After proper hand washing, the skin over the venepuncture site is disinfected with povidone solution and 70% ethyl alcohol. The flip-off cap in the neonatal blood culture bottle is removed and disinfected with spirit. With strict aseptic precautions 2 ml of blood is collected from the neonates and with the same syringe the blood is transferred into the blood culture bottle with 20 ml of BHI broth[4,5]. The blood culture bottle is immediately transported to the Microbiology laboratory and it should be never refrigerated. It is incubated at 37 degree C and daily examined for the presence of bacterial growth. Bacterial growth is indicated by the presence of turbidity in the broth and hemolysis. Subcultures are made from the broth daily for first three days and then alternate days upto seven days. Subcultures are made in blood agar and MacConkey agar. Bacterial isolates are identified by colony characteristics, Gram staining, growth on special media and biochemical properties like catalase and oxidase test, coagulase test, motility, IMViC test, urease test, TSI agar test. Antibiotic susceptibility testing is done by Kirby Bauer method with specific antibiotic discs. Bacterial inoculum is compared with 0.5 McFarland standard and lawn culture is streaked on Muller Hinton agar[6,7]. The results are interpreted as per CLSI guidelines. ATCC strains are used as standard strains.

Blood culture samples

Total	Culture positive N(%)	Culture negative N(%)
200	88(44%)	112(66%)

Culture positive organisms

Total	Males N(%)	Females N(%)
88	51(57.95%)	37(22.1%)

Gram positive organisms

Total	*Staphylococcus aureus* N(%)	CONS N(%)
66	48(72.73%)	18(37.5%)

Culture positive organisms

Total	Gram positive N(%)	Gram negative N(%)
88	66(75%)	22(25%)

Gram negative organisms

Total	*E.coli* N(%)	*Klebsiella sp.* N(%)	*Pseudomons sp.* N(%)
22	12(54.5%)	8(36.36%)	2(9.09%)

Quality control

Uninoculated blood culture bottle is also incubated and looked for any bacterial growth.

Data analysis

Chi-square test was used. P value of 0.05 or less than that was considered as statistically significant.

Antibiotic discs used

Gram positive organisms

Oxacillin(5 µg), erythromycin(15 µg), vancomycin(30 µg), linezolid(30 µg), gentamycin(10 µg), amikacin(30 µg), ciprofloxacin(5 µg), clindamycin(10 µg), chloramphenicol(30 µg) and teicoplanin(30 µg).

Table 1. Drug sensitivity pattern for Gram positive organisms

Drug	*Staphylococcus aureus* (48) N(%)	CONS(18) N(%)
Oxacillin	5(10.41)	2(11.2)
Erythromycin	4(8.3)	2(11.2)
Vancomycin	39(81.25)	15(83.33)
Linezolid	45(93.75)	17(94.5)
Gentamycin	15(31.25)	4(32.3)
Amikacin	39(81.25)	15(83.4)
Ciprofloxacin	25(52.1)	10(65.6)
Clindamycin	39(81.25)	15(83.4)
Chloramphenicol	25(52.1)	10(55.55)
Teicoplanin	42(87.5)	15(83.4)

Gram negative organisms

ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), Cefotaxime (30 µg), Cefoperazone (75 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg),nalidixic acid (30 µg), Ampicillin (10 µg), Piperacillin (100 µg) and chloramphenicol (30 µg).

RESULTS

Out of 200 neonatal blood culture samples, 88 samples (44%) showed positive blood culture. In the positive blood culture samples, 51 were males(57.95%)and 37(42.05%) were females[8].

Neonates presented with fever, respiratory distress, tachycardia, refusal of feeds and jaundice.

In the positive blood culture samples, 66(75%) were Gram positive organisms and 22(25%) were Gram negative organisms. Among the Gram positive organisms, Staphylococcus aureus were isolated in 48 samples (72.72%) and CONS were isolated in 18 samples(27.27%). Among the Gram negative organisms, *E.coli* were isolated in 12 samples (54.55%)and *Klebsiella* sp. were isolated in 8 samples(36.36%) and *Pseudomonas* sp. were isolated in 2 samples (9.1%). CONS were considered as pathogenic organism if there was history of prior antibiotic administration, the body temperature of the neonate was one degree more than or less than 37 degree or prolonged intravenous catheterization[9,10].

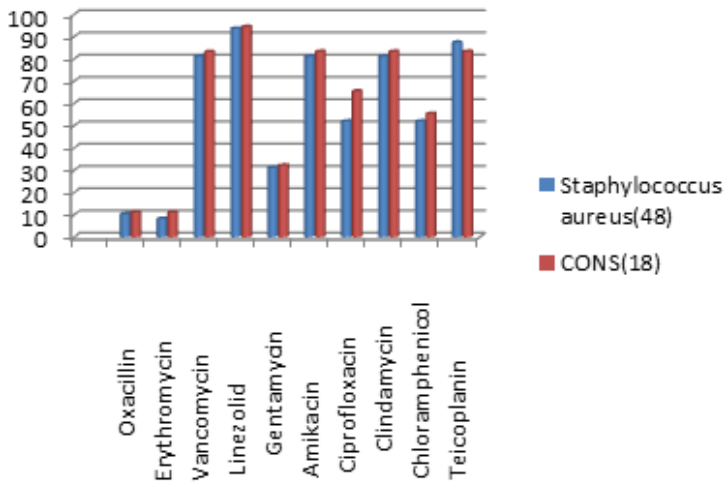

Fig.1. Comparison of Drug sensitivity pattern for Gram positive organisms

DISCUSSION

Neonatal bacteremia is difficult to diagnose clinically as the presentation is mostly nonspecific. Blood culture remains the gold standard method for the lab diagnosis.

The common neonatal risk factors in this study were prematurity, low birth weight and prolonged intravenous catheterisation. Immature immune system may be the reason for the occurrence of bacteremia in premature and low birth weight neonates. Maternal risk factors include genitourinary infections and premature rupture of membranes[11].

Bloodstream infections in early neonatal period may be of maternal origin like early rupture of membranes or genitourinary infections. Bacteremia in late neonatal period is mostly

Table 2. Drug resistance pattern for Gram positive organisms

Drug	*Staphylococcus aureus* (48) N(%)	CONS(18) N(%)
Oxacillin	43(89.6)	16(88.88)
Erythromycin	44(91.66)	16(88.88)
Vancomycin	9(37.5)	3(16.7)
Linezolid	3(6.25)	1(5.55)
Gentamycin	33(68.75)	14(77.77)
Amikacin	9(18.75)	3(16.66)
Ciprofloxacin	23(47.9)	8(44.44)
Clindamycin	9(18.75)	3(16.66)
Chloramphenicol	23(47.92)	8(16.6)
Teicoplanin	6(12.5)	3(16.6)

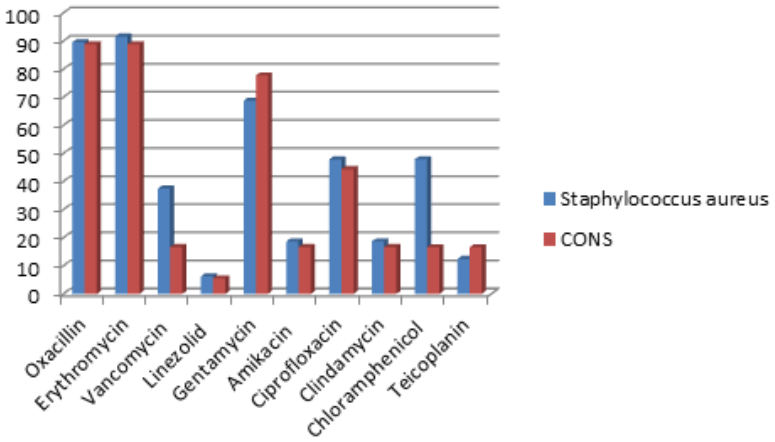

Fig. 2. Comprison of Drug resistance pattern for Gram positive organisms

Table 3. Drug sensitivity pattern for Gram negative organisms

Drug	*E.coli* (12) N(%)	*Klebsiella sp.*(8) N(%)	*Pseudomonas sp.*(2) N(%)
Ciprofloxacin	10(83.3)	6(75)	1(50)
Gentamycin	3(25)	5(62.5)	1(50)
Amikacin	11(91.66)	7(87.5)	2(100)
Cefotaxime	3(25)	1 (12.5)	(0)
Cefoperazone	1(8.33)	2(25)	1(50)
Cotrimaxazole	1(8.33)	1(12.5)	(0)
Nalidixic acid	4(33.33)	5(62.5)	1(50)
Ampicillin	3(25)	2(25)	(0)
Chloramphenicol	2(16.66)	1(12.5)	(0)
Piperacillin	10(83.33)	6(75)	2(100)

community acquired or hospital acquired. Neonates with prolonged stay at NICU are exposed to the hospital flora. Skin and mucosal carriers of hospital personnel and baby handlers may transmit the infections to the neonates. Staphylococcus epidermidis may cause bloodstream infections because of biofilm formation in intravenous catheters. Skin damage occurring during catheter insertion inhibits TLR2-dependent pro-inflammatory cytokine production and this will allow *S. epidermidis* to adhere, proliferate and migrate along catheters with minimum interference from host defenses[12]. This is common in preterm neonates. Lipoteichoic acid and lipid S present in Staphylococcus cell wall has got antiphagocytic properties. This may be the reason for high occurrence of Gram positive organisms in neonatal bloodstream infections[13].

Neonates presented with fever, respiratory distress, refusal of feeds and jaundice.

In this study, out of 200 neonatal blood culture samples, 88 (44%) were blood culture positive.This percentage was comparable to other studies like Roy *et al* and Kayange *et al*.

In the present study 47% were low birth weight neonates and 35% were preterm neonates in the positive blood culture samples. This may be because of the immature immune system in these neonates[14].

In the positive blood culture samples, the percentage of males (57.95%) was higher than females (42.05%).This was comparable to other

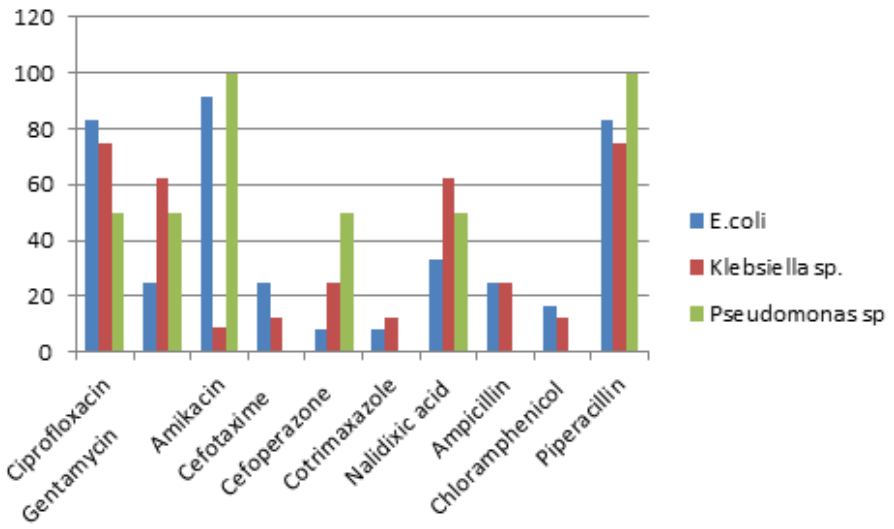

Fig.3. Comparison of various Drug sensitivity pattern for Gram negative organisms

Table 4. Drug resistance pattern of Gram negative organisms

Drug	*E.coli* (12) N(%)	*Klebsiella sp.*(8) N(%)	*Pseudomonas sp.*(2) N(%)
Ciprofloxacin	2(16.6)	2(25)	1(50)
Gentamycin	9(75)	3(37.5)	1(50)
Amikacin	1(8.33)	1(12.5)	- (0)
Cefotaxime	9(75)	7(87.5)	2(100)
Cefoperazone	11(91.66)	6(75)	1(50)
Cotrimaxazole	11(91.66)	7(87.5)	2(100)
Nalidixic acid	8(66.66)	3(37.5)	1(50)
Ampicillin	9(75)	6(75)	2(100)
Chloramphenicol	10(83.33)	7(87.5)	2(100)
Piperacillin	2(16.66)	2(25)	(0)

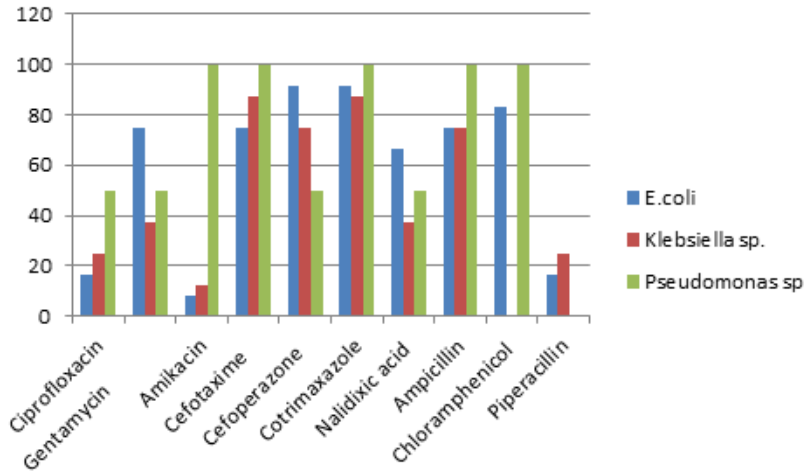

Fig. 4. Comparison of Drug resistance pattern of Gram negative organisms

studies like Begum *et al* and Sreshtha *et al*. This may be due to X linked gene regulation of gamma globulin synthesis. The figures 1,2,3 and 4 shows the comparison of different types of drugs with gram positive and negative organism with graphical representation.

The tabular 1,2,3 and 4 are shows the isolation rate of Gram positive organisms (75%) was higher than Gram negative organisms (25%). This is in concordance with other studies like Ballot *et al* ,Kaufman and Fairchild and Hoogen *et al*. Among the Gram positive organisms, *Staphylococcus aureus* was the predominant isolate (72.73%) followed by CONS(27.3%).This may be due to colonization of skin and mucosa in hospital personnel's and improper hand washing techniques. Among the Gram negative organisms, the isolation rate of *E.coli* was 54.5% followed by *Klebsiella* sp.(36.36%) and *Pseudomonas* sp. was isolated in 9.09%[15,16].

Most of the Gram positive organisms showed oxacillin resistance. *Staphylococcus aureus* showed 89.6% and CONS showed 88.88% oxacillin resistance. They also showed high resistance to erythromycin, gentamycin, ciprofloxacin and chlramphenicol. Vancomycin resistance rate was 18.7% for *Staphylococcus aureus* and 16.7% for CONS. In this study, *Staphylococcus aureus* was sensitive to clindamycin in 81.25% and amikacin in 81.25%.It showed the highest sensitivity towards teicoplanin and linezolid. CONS also showed similar sensitivity pattern[17].

Gram negative isolates like *E.coli* and *Klebsiella* sp. showed higher resistance rate towards gentamycin (37.5-75%), third generation cephalosporins (75-95.66%), nalidixic acid(37.5-75%) and chloramphenicol(83.33-87.5%)[18].The sensitive rate of *E.coli* for amikacin was 91.66% and for piperacillin was 83.33%. *Klebsiella* sp. showed higher sensitivity to amikacin (87.5%). *Pseudomonas* sp. was resistant to gentamycin(50%), ciprofloxacin(50%), cefaperazone(50%) and cefataxime(100%). It showed 100% sensitivity towards piperacillin. The higher resistance rate of organisms may be due to injudicious use of antibiotic drugs and bacterial mechanisms of drug resistance[19].

CONCLUSION

In this study Gram positive organisms were the predominant isolates in blood stream infections in neonates. Staphylococcus aureus and CONS showed higher resistance rates towards oxacllin. Gram negative organisms showed higher resistant rate towards third generation cephalosporins and gentamycin. Gram negative organisms were more sensitive towards amikacin and piperacillin. Proper hand washing and treatment of Staphylococcal carriers will reduce the

incidence of blood stream infections in neonates. Proper selection of antibiotic drugs after blood culture and antibiotic susceptibility testing should be implemented and infection control policy should be formed to reduce bacteremia in neonates.

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