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Original Article

Bacterial Agents and their Antibiotic Resistance Pattern in Neonatal Blood Cultures - A Hospital Based Study

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Abstract

Introduction: Bacterial infections are an important cause of neonatal mortality and morbidity worldwide. Despite advances in neonatal care, antibiotic resistance among common pathogens and the emergence of multidrug-resistant organism continues to be a challenge in the neonatal intensive care units (NICU) today.

Methods: This retrospective hospital based study was conducted over a period of 2 years. We analyzed the results of neonatal blood cultures, their bacteriological profile and the antibiotic resistance pattern.

Results: Out of 1084 neonates screened for sepsis there were 241 (22.23%) positive blood cultures. After excluding coagulase-negative *Staphylococcus* and *Flavobacterium* which were suspected to be skin contaminants and also fungi, we analyzed 52 cases of bacterial growth. The predominant growth was Gram-positive organisms (51.92%), of which *Staphylococcus aureus* and *Enterococcus* were the predominant isolates. The Gram-negative organism constituted 48.08%, of which *Acinetobacter* (19.23%) was the most common followed by coliforms (13.46%). The Gram-positive organisms had high resistance to penicillin and ampicillin but were highly sensitive to vancomycin, linezolid, and netilmicin. The Gram-negative group showed high resistance to ampicillin and quinolones and high susceptibility to netilmicin, amikacin, and meropenem.

Conclusion: Periodic surveillance of the bacterial agents and understanding their antibiotic resistance pattern will definitely help in formulating rational antibiotic practices in the NICU.

Key words: Antibiotic resistance, Bacterial agents, Blood cultures, Gram-negative organism, Neonatal sepsis

INTRODUCTION

Neonatal sepsis is a clinical syndrome of bacteremia with systemic signs and symptoms of infection accompanied by positive blood culture in the first 28 days of life.[1] Despite advances in Newborn care neonatal sepsis is still a leading cause of morbidity and mortality, especially in low birth weight and preterm babies.[2,3] Neonatal septicemia can be divided into early-onset sepsis (EOS) and late-onset sepsis (LOS) depending on the age of presentation. The usual organism and the method of transmission are different in the two groups. EOS (first 72 h of birth) is due to vertical transmission during labor whereas LOS (after 72 h) is due to vertical, horizontal, or nosocomial infection. The incidence and organisms causing neonatal septicemia varies geographically, and also over time.[4] Group B *Streptococci*, *Escherichia coli,* and *Listeria monocytogenes* predominate in developed countries whereas *Staphylococci* and Gram-negative bacilli are much more common in developing countries.[5] Although morbidity and mortality due to neonatal sepsis have decreased over recent years, studies report widespread drug resistance to the commonly used antibiotics.[6] Knowledge of the common organisms and their antibiotic resistance will help in early initiation of appropriate therapy consistent with successful treatment and improvement in outcome.

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This study was conducted to determine the common bacterial pathogens, and their antibiotic resistance pattern from blood cultures of neonates admitted for sepsis at neonatal intensive care unit (NICU), Government Medical College, Ernakulum, Kerala.

MATERIALS AND METHODS

This is a descriptive study conducted in the NICU of Government Medical College Ernakulam over a period of 2 years (January 2014 - December 2015). We retrospectively evaluated the case records of babies whose blood cultures were taken as part of screening for both early and late onset neonatal sepsis during the study period.

Blood samples collected from babies at risk for sepsis on the basis of maternal fever, maternal urinary tract infection, prolonged rupture of membranes more than 24 h, foul smelling liquor, or babies with symptoms of lethargy, poor feeding, tachypnea, abdominal distention, feed intolerance, tachycardia, hypothermia, apnea, and seizures suggestive of sepsis were included in the study. Neonates with gross congenital anomalies and those who received antibiotics before taking blood culture were excluded from the study.

Blood cultures were drawn under strict aseptic precaution before starting antibiotics. 2 ml of blood was inoculated in 20 ml of brain heart infusion broth. This was further incubated for the next 7 days at 37°C. Subcultures were done on blood agar and MacConkey's agar after assessing whether the broth was clear or turbid. If it was turbid, then it was subcultured immediately and if it remains clear, sub cultured after 48 h. If there is no growth, further incubated for next 7 days and sub cultured on the 7th day. If there was no bacterial growth after 7 days of incubation, the culture was reported to be negative.[7] If growth was present, organisms were identified on the basis of colony morphology and standard biochemical tests as per standard lab protocol.[7]

The sensitivity of bacteria to different antibiotics including ampicillin, amikacin, gentamicin, cefalotin, levofloxacin, meropenem, vancomycin, and clindamycin was investigated according to the standard disk diffusion (Kirby-Bauer) method recommended by Clinical and Laboratory Standards Institute.[8] The demographic data, blood culture reports, bacterial isolates and their antibiotic resistance pattern were obtained from the unit register and neonatal case records. Data were analyzed using Statistical Package for Social Sciences version 16 software.

RESULTS

There were 241 (22.23%) positive blood cultures (Table 1) out of 1084 neonates screened for neonatal sepsis. Growth of Coagulase-negative *Staphylococci* (184 cases), other possible skin contaminants which included *Flavobacterium* (3 cases) and fungi (2 cases) were excluded. The remaining 52 cases of neonatal sepsis with bacterial growth were evaluated further for antibiotic resistance pattern (Table 2). Out of 1084 neonates included in the study, male (628) to female (456) ratio was 1.38:1 and term (604) to preterm (480) ratio was 1.26:1.

The predominant growth was Gram-positive organisms (51.92%), of which *Staphylococcal aureus* and *Enterococcus* (15.38%) were the predominant isolates followed by *Corynebacterium* (11.54%) and *Streptococcus viridans* (9.61%).

Of the Gram-negative group, *Acinetobacter* (19.23%) was the most common followed by coliforms (13.46%). The other Gram-negative isolates included *Pseudomonas* (7.69%), *Klebsiella* (5.77%), and *Neisseria* (1.92%).

In the Gram-positive group, complete sensitivity was seen to linezolid, netilmicin and teicoplanin (100%) followed by vancomycin (93.33%) and gentamicin (56.52%) while complete resistance was observed to penicillin and ampicillin. *S. aureus* showed 100 % resistance to penicillin and 100% sensitivity to netilmicin while *Enterococci* showed 100% resistance to cephalosporins and ampicillin. Complete susceptibility to linezolid was seen in both organisms (Figure 1).

In the Gram-negative group, high resistance was noted to ampicillin (95%) followed by oxacillin (76.19%) and piperacillin (71.43%). High sensitivity was noted to meropenem (88.24%) followed by netilmicin (77.78%). *Acinetobacter* which was the predominant isolate showed high sensitivity to gentamicin (80%) followed by

Table 1: Microbiological profile of isolates

Blood culture	*n* (%)
Total samples	1084
Positive culture	241 (22.23)
CONS	184
Bacterial growth excluding CONS	52 (100)
Acinetobacter	10 (19.23)
Coliform	7 (13.46)
Corynebacterium	6 (11.54)
Enterococcus (Strep D)	8 (15.3)
Streptococcal viridans	5 (9.62)
Klebsiella	3 (5.77)
Neisseria	1 (1.92)
Pseudomonas	4 (7.69)
Staphylococcus aureus	8 (15.38)

CONS: Coagulase-negative *Staphylococcus*

Table 2: Resistance and sensitivity pattern of organisms

Antibiotic	Gram-positive resistance	Gram-positive sensitive	Gram-negative resistance	Gram-negative sensitive
Penicillin	27 (100)	0	NT*	NT
Gentamicin	10 (43.48)	13 (56.52)	11 (44)	14 (56)
Linezolid	0	21 (100)	NT	NT
Netilmicin	0	8 (100)	4 (22.22)	14 (77.78)
Vancomycin	1 (6.67)	14 (93.33)	NT	NT
Cefalotin	14 (77.78)	4 (22.22)	16 (64)	9 (36)
Oxacillin	6 (42.86)	8 (57.14)	16 (76.1)	5 (23.81)
Teicoplanin	0	8 (100)	NT	NT
Levofloxacin	NT	NT	8 (36.36)	14 (63.64)
Meropenem	NT	NT	2 (11.76)	15 (88.24)
Piperacillin	NT	NT	5 (71.43)	2 (28.57)
Ampicillin	27 (100)	0	19 (95)	1 (5)
Amikacin	NT	NT	11 (44)	14 (56)
Clindamycin	12 (57.14)	9 (42.86)	NT	NT

*NT: Not tested

cefoperazone (70%) and complete resistance to ampicillin followed by amikacin (50%). *Pseudomonas* showed high resistance to ceftazidime (75%) and piperacillin (75%) and complete sensitivity to netilmicin and amikacin. *Klebsiella* showed 100% resistance to piperacillin, gentamicin and amikacin and 67% sensitivity to meropenem and netilmicin. Sensitivity of coliforms to meropenem and amikacin was 100% and 71.42%, respectively (Figure 2).

All Gram-positive organisms showed high resistance to ampicillin, penicillin, and high sensitivity to vancomycin, linezolid, and netilmicin. Gram-negative organisms had high resistance to ampicillin, piperacillin, and quinolones and were highly sensitive to amikacin, netilmicin, and meropenem.

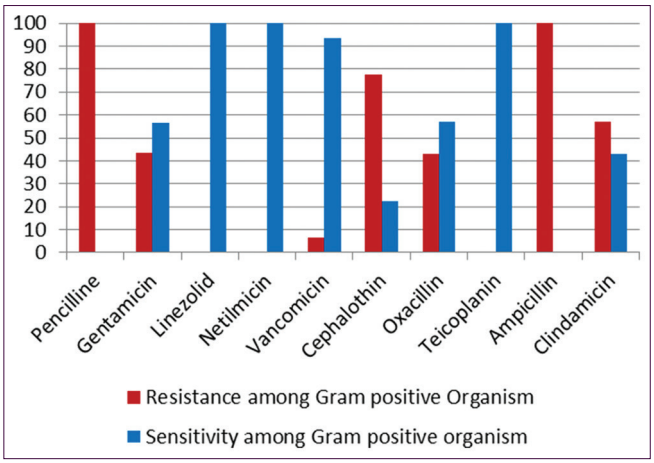

Figure 1: The antibiotic resistance pattern (%) of Gram positive bacteria

DISCUSSION

In this study Gram-positive organisms (51.92%) dominated over Gram-negative organisms (48.08%) which correlated with various other studies.[9-11] However, some studies have found the frequency of isolation of Gram-negative organism more than Gram-positive organisms.[12-14]

Acinetobacter was the predominant organism isolated in our study (19.23%). It is important to highlight the fact that over the recent years *Acinetobacter* has been isolated as an important pathogen in neonatal sepsis. Incidence of *Acinetobacter* reported by Shete *et al.* from India and Shamsul *et al.* from Nepal was 10.8% and 9.5%, respectively[15,16] whereas Jarousha *et al.* from Iran reported the incidence to be 6.9%.[17] Increasing rates of *Acinetobacter* infections may be due to lapses in infection control and prolonged hospitalizations in preterm infants.[18] *S. aureus* and *Enterococcus* were also significant organisms in our study (15.38%). This pattern slightly differed from the findings from various other studies where *Klebsiella* and

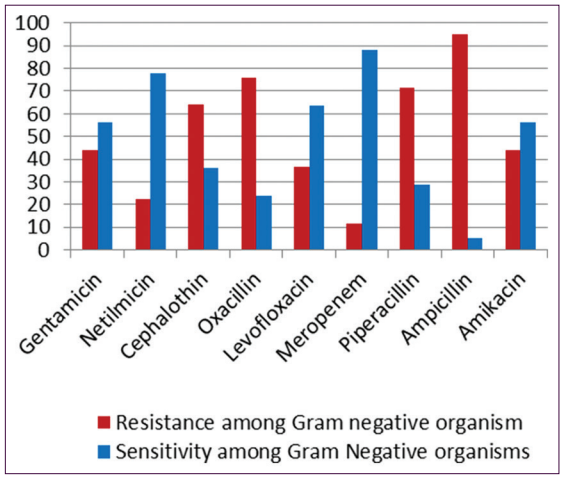

Figure 2: The antibiotic resistance pattern (%) of Gram negative bacteria

S. aureus were the predominant organisms.[19-21] Recent data from Pakistan and India reveals that *S. aureus*, *Klebsiella,* and *E. coli* are the usual organisms in neonatal units, with high incidence of multi-drug resistance.[22,23] *Enterococcus* is

also another pathogen in serious nosocomial infection in newborns. The incidence of the organisms causing sepsis has increased three folds over the recent years.[24]

In our study, *Acinetobacter* species was completely resistant to ampicillin (100%). They were more sensitive to gentamicin and cefoperazone (70%). Najeeb *et al.*[25] and Jeyamurugan *et al.*[19] also described high resistance of these bacteria against ampicillin and commonly used antibiotics. Multidrug-resistant *Acinetobacter* is also of growing concern in neonatal sepsis.[26] Among other Gram-negative isolates which included coliforms, *Klebsiella,* and *Pseudomonas* the best sensitivity was observed to meropenem (92.85%) and netilmicin (78.57%). Sensitivity to amikacin and gentamicin were almost similar (55.5%) and (64%). High resistance was noted to ampicillin (88.8%). The findings were similar to a study conducted by El-Din *et al.*[27] from Egypt where they observed high sensitivity to meropenem and quinolones, intermediary resistance to gentamicin and amikacin and complete resistance to ampicillin.

In the Gram-positive group, complete sensitivity was seen to linezolid, netilmicin, and teicoplanin (100%) followed by vancomycin (93.33%) and gentamicin (56.52%) while complete resistance was observed to penicillin and ampicillin. This pattern of resistance was similar to studies conducted in Nepal,[15] Gujarat,[28] and Kanpur.[29] In our study, *Staphylococcus* was completely sensitive to vancomycin though methicillin resistance was noted in 25%.

CONCLUSION

This study identified *Acinetobacter*, *S. aureus*, and *Enterococcus* and coliforms as the predominant causative agents of neonatal sepsis in our unit, of which the emergence of *Acinetobacter* is of concern. The alarmingly high resistance pattern to penicillin which is used as first-line antibiotic in our unit should be seriously considered when formulating antibiotic policy for empirical therapy. Various antibiotic combinations and the usage of newer antibiotics should also be judicious. Strict infection control measures in the NICU still stands the best along with constant monitoring and surveillance of neonatal bloodstream infection to avoid the emergence of multidrug-resistant organisms.

Limitations of our study include its retrospective nature, and similar set of antibiotics were not tested always for sensitivity of organisms.

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