Descriptive Study to Find out Antibiotic Susceptibility Pattern of the Clinical Isolates of Neonatal Septicemia

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A B S T R A C T

The most common causes of death in neonatal period are infections (32%) including septicemia, meningitis, pneumonia, diarrhea and neonatal tetanus. It has also been documented that birth asphyxia contributes for 29% of deaths in neonatal period. Patients presented to department of pediatrics (NICU), were examined clinically by pediatricians and 122 cases of neonatal septicemia were identified on the basis of the signs and symptoms and were included for the study. This is followed by collection of blood for culture after obtaining informed expressed written consent.GPCs were predominantly susceptible to vancomycin and clindamycin whereas GNBs were predominantly susceptible to imipenem, eftazidime/clavulanic acid and chloramphenicol.

Keywords
Antibiotic Susceptibility, Clinical Isolates, Neonatal Septicemia

Introduction

Reported incidence of neonatal deaths is of about 5 million in a year according to the estimation of World Health Organization (WHO), (Rasul et al., 2007) among these 5 million deaths, it is observed that about 98% are occurring in developing countries within first week of life. Moreover, death rate widely varies in the developing countries between 11-68/1000 live births in Asia, Africa and Latin America. It is assumed that neonatal mortality in the developing countries is under reported by at least 20%. The most common causes of death in neonatal period are infections (32%) including septicemia, meningitis, pneumonia, diarrhea and neonatal tetanus. It has also been documented that birth asphyxia contributes for 29% of deaths in neonatal period. (Bang et al., 2001)

Fetus is protected from the microbial flora of the mother by chorioamniotic membrane, the placenta and presence of anti-bacterial in amniotic fluid. Bacterial colonization of the neonate takes place after rupture of the maternal membranes. If the rupturing of the membranes lasts longer than 24 hours, vaginal bacteria may ascend and in some cases produce inflammation of the fetal membranes,
Umbilical cord and placenta (Wientzen and McCracken, 1977). Neonates have relatively immature immune defense mechanisms. (Frank, 1979) But passively transferred specific IgG antibody from the mother in adequate concentration provides neonate protection against infection. The average concentration of IgG in a preterm infant is 400mg/dl and in term infant it is 1000mg/dl. Since IgM and IgA antibodies are not transplacentally transferred from the mother, the foetus is able to synthesize pathogen specific antibodies, for instance, IgM and IgA antibodies in response to intrauterine infection but the production is inadequate. This gives us the reason for increased susceptibility of newborn to infections. Furthermore, lack of transfer of complement also contributes for enhanced susceptibility of the newborn to infections with GNBs. Newborn once infected, there is rapid multiplication and spread of infection to different organs through bloodstream producing various systemic manifestations.

Leucopenia generally is observed in neonates. Production of T cells and cytokine production by macrophages are also seen in decreased amounts. Macrophage and natural killer cell functions are decreased leading to impaired phagocytosis and delay in response to infection.

The treatment includes supportive therapy, antibiotic therapy and adjunctive therapy. Neonatal septicemia is a life threatening emergency and accurate treatment with antibiotics is essential for a favourable outcome. Neonate with septicemia needs to be given basic supportive care which includes providing of thermo neutral environment, oxygen supplementation along with ventilator support when required, regular monitoring of patient to keep in check of hypo glycaemia and hyper glycaemia. However, maintenance of blood pressure and normal tissue perfusion is done with colloides and ionotropes. In case of anaemia and bleeding diathesis, correction can be done by utilizing packed cells and fresh frozen plasma (Wientzen and McCracken, 1977)

Prior to the antibiotic era, the mortality attributed to septicemia was 90% but it declined to 24-58% after antibiotic came into use. (Gupta et al., 1989) The empiric antimicrobial approach for neonatal sepsis has changed in many centres. In a study conducted by Mustafa and Ahmed it was observed that GPCs were susceptible to amikacin, cephalosporins and ciprofloxacin with higher susceptibility to linezolid and vancomycin. However, they were recorded more resistant to ampicillin and gentamicin. But higher resistance was documented for GNBs to commonly used antibiotics. (Agnihotri et al., 2004) In their retrospective study documented S.aureus including GNBs as resistant to amoxicillin, most of the strains of S.aureus being more sensitive to netilmicin. The authors (Agnihotri et al., 2004) concluded aminoglycosides, 3rd generation cephalosporins and quinolones as the most suitable drugs for the treatment of neonatal septicemia. Appropriate antibiotic usage has been found effective in reducing resistance in few clinical settings. Hand washing has consistently shown reduction in incidence of nosocomial neonatal sepsis.

Materials and Methods

Inclusion criteria

Clinically suspected cases of neonatal septicemia.

Exclusion criteria

Neonates clinically suspected of septicemia but had received antibiotics were excluded from the study.
Patients presented to department of pediatrics (NICU), were examined clinically by pediatricians and 122 cases of neonatal septicaemia were identified on the basis of the signs and symptoms and were included for the study. This is followed by collection of blood for culture after obtaining informed expressed written consent.

**Antibiotic susceptibility testing**

The clinical isolates obtained were subjected to antibiotic susceptibility testing by disc diffusion technique to determine the susceptibility pattern following the standard procedure. Briefly, bacterial inoculum for antibiotic susceptibility testing was prepared from 4–5 well-isolated colonies in nutrient broth medium. The broth culture was incubated at 37°C. Suspension of the growing bacterium in broth medium with a turbidity equivalent to that of 0.5 McFarland standard was cultured using a sterile swab over Mueller–Hinton agar by evenly spreading in three directions over the entire surface of the medium so as to obtain uniform growth.

Appropriate antibiotic discs (HIMEDIA, Mumbai, India) were applied maintaining a distance of 24 mm between 2 adjacent discs and the plates were incubated at 37°C after placing these discs. Simultaneously the test was carried out with standard bacterial strains of *S.aureus* ATCC 29213, *E.coli* ATCC 25922 and *P.aeruginosa* ATCC 27853. Clinical isolates indicating GPCs were tested for antibiotics such as amikacin, ampicillin, cefazolin, cefotaxime, ceftriaxone, ceftazidime, ceftazidime/clavulanic acid (when found resistance to ceftazidime), cefuroxime, ciprofloxacin, chloramphenicol, cotrimoxazole, erythromycin, gentamicin, imipenem, netilmicin, ofloxacin. After 16-18 hours of incubation, the plates were examined and the diameter of the zone of complete inhibition around the discs was measured by a ruler. The diameter of zone of inhibition for individual antimicrobial agent was considered to state as sensitive or resistant by referring to an interpretative chart for the Kirby-Bauer method as per the recommendation of the CLSI. The antibiotic discs for the study were purchased from HIMEDIA, Mumbai, India.

Information regarding demographic characteristics like age and sex of patients, clinical manifestations including the risk factors, physical examination findings, microbiological data of the clinical isolate and other relevant laboratory parameters were all entered in the proforma and analyzed.

**Results and Discussion**

As depicted in tables 1 and 2, among 47 clinical isolates tested for antibiotic sensitivity, the pattern observed was - GPCs were predominantly susceptible to vancomycin and clindamycin whereas GNBs were predominantly susceptible to imipenem, ceftazidime/clavulanic acid and chloramphenicol.
Table 1: Spectrum of antibiotic sensitivity pattern of 26 clinical isolates of GPCs

| Antibiotic          | No. (%) of MRSA strains sensitive to [out of 6 isolates tested] | No. (%) of S. aureus isolates sensitive to [out of 8 isolates tested] | No. (%) of CONS isolates sensitive to [out of 9 isolates tested] | No. (%) of Streptococcus isolates sensitive to [out of 2 isolates tested] | No. (%) of Pneumococcus isolate sensitive to [out of 1 isolate tested] |
|---------------------|---------------------------------------------------------------|---------------------------------------------------------------------|------------------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------|
| Ampicillin          | 3(50)                                                        | 1(12.50)                                                            | 4(44.44)                                                        | 0(00)                                                                    | 0(00)                                                                 |
| Cefazolin           | 2(33.33)                                                     | 4(50)                                                              | 2(22.22)                                                        | 1(50)                                                                    | 0(00)                                                                 |
| Cefotaxime          | 2(33.33)                                                     | 1(12.50)                                                            | 2(22.22)                                                        | 0(00)                                                                    | 0(00)                                                                 |
| Ceftazidime         | 2(33.33)                                                     | 1(12.50)                                                            | 1(11.11)                                                        | 0(00)                                                                    | 0(00)                                                                 |
| Cefuroxime          | 4(66.66)                                                     | 5(62.50)                                                            | 2(22.22)                                                        | 0(00)                                                                    | 0(00)                                                                 |
| Ciprofloxacin       | 3(50)                                                        | 5(62.50)                                                            | 2(22.22)                                                        | 1(50)                                                                    | 1(100)                                                                |
| Clindamycin         | 6(100)                                                       | 8(100)                                                              | 2(22.22)                                                        | 2(100)                                                                    | 0(00)                                                                 |
| Chloramphenicol     | 5(83.33)                                                     | 8(100)                                                              | 2(22.22)                                                        | 1(50)                                                                    | 1(100)                                                                |
| Cotrimoxazole       | 3(50)                                                        | 6(75)                                                               | 2(22.22)                                                        | 1(50)                                                                    | 1(100)                                                                |
| Erythromycin        | 0(00)                                                        | 1(12.50)                                                            | 2(22.22)                                                        | 0(00)                                                                    | 0(00)                                                                 |
| Gentamicin          | 2(33.33)                                                     | 5(62.50)                                                            | 2(22.22)                                                        | 0(00)                                                                    | 0(00)                                                                 |
| Netilmicin          | 5(83.33)                                                     | 6(75)                                                               | 2(22.22)                                                        | 1(50)                                                                    | 1(100)                                                                |
| Ofloxacin           | 5(83.33)                                                     | 3(37.50)                                                            | 2(22.22)                                                        | 1(50)                                                                    | 1(100)                                                                |
| Penicillin G        | 0(00)                                                        | 1(12.50)                                                            | 3(33.33)                                                        | 0(00)                                                                    | 0(00)                                                                 |
| Teicoplanin         | 0(00)                                                        | 1(12.50)                                                            | 3(33.33)                                                        | 0(00)                                                                    | 0(00)                                                                 |
| Vancomycin          | 6(100)                                                       | 8(100)                                                              | 9(100)                                                          | 2(100)                                                                    | 1(100)                                                                |

Table 2: Spectrum of antibiotic sensitivity pattern of 21 clinical isolates of GNBs

| Antibiotic                  | No. (%) of K. aerogenes isolates sensitive to [out of 14 isolates tested] | No. (%) of P. aeruginosa isolate sensitive to [out of 1 isolate tested] | No. (%) of C. freundii isolate sensitive to [out of 1 isolate tested] | No. (%) of E. coli isolate sensitive to [out of 4 isolates tested] | No. (%) of Acinetobacter isolates sensitive to [out of 4 isolates tested] |
|-----------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Ampicillin                  | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Cefazolin                   | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Cefoxime                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Cefotaxime                  | 2(14.28)                                                                | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Ceftazidime                 | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Ceftazidime/Clavulanic acid| 10(71.42)                                                                | 1(100)                                                                  | 1(100)                                                                  | 1(100)                                                                  | 4(36.36)                                                                |
| Chloramphenicol             | 11(78.57)                                                                | 1(100)                                                                  | 1(100)                                                                  | 1(100)                                                                  | 3(27.27)                                                                |
| Cefuroxime                  | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 2(18.18)                                                                |
| Ciprofloxacin               | 8(57.14)                                                                | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Cotrimoxazole               | 10(71.42)                                                                | 0(00)                                                                    | 1(100)                                                                  | 0(00)                                                                    | 3(27.27)                                                                |
| Erythromycin                | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Gentamicin                  | 5(35.71)                                                                | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Imipenem                    | 13(98.85)                                                                | 0(00)                                                                    | 1(100)                                                                  | 1(100)                                                                  | 2(18.18)                                                                |
| Netilmicin                  | 2(14.28)                                                                | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    | 0(00)                                                                    |
| Ofloxacin                   | 3(21.42)                                                                | 0(00)                                                                    | 1(100)                                                                  | 0(00)                                                                    | 1(9.09)                                                                  |
Table 3 Antibiotic susceptibility pattern of GPCs in different studies

| Authors and reference | Region              | Most sensitive antibiotic/s against Gram positive cocci | Antibiotic/s to which most Gram positive cocci were resistant |
|-----------------------|---------------------|--------------------------------------------------------|---------------------------------------------------------------|
| Tallur et al., (2000) | Hubballi, Karnataka | Gentamicin Amikacin Tobramycin                         | Ampicillin                                                    |
| Kaistha et al., (2009) | Chandigarh          | Vancomycin (100%)                                       | Gentamicin (50%) Erythromycin (46.5%)                         |
| Aletayeb et al., (2011)| Ahvaz, Iran         | Vancomycin (100%)                                       | Ampicillin (100%) Penicillin (100%)                           |
| Present study         | Belagavi, Karnataka | Vancomycin (100%) Clindamycin (69.23%)                 | Erythromycin (88.46%) Teicoplanin (84.61%) PenicillinG (84.61%) |

Table 4 Antibiotic susceptibility pattern of GNBs in different studies

| Authors and reference | Region              | Most sensitive antibiotic/s against Gram negative bacilli | Antibiotic/s to which most Gram negative bacilli were resistant |
|-----------------------|---------------------|----------------------------------------------------------|---------------------------------------------------------------|
| Tallur et al., (2000) | Hubballi, Karnataka | Gentamicin Amikacin Tobramycin                         | Ampicillin                                                    |
| Kaistha et al., (2009)| Chandigarh          | Amikacin Imipenem                                        | Amoxycillin (62%) Ampicillin (83%)                            |
| Aletayeb et al., (2011)| Ahvaz, Iran         | Imipenem (100%)                                          | Ampicillin (100%) Gentamicin (100%)                           |
| Present study         | Belagavi, Karnataka | Imipenem (80.95%) Ceftazidime/Clavulanicacid (80.95%) Chloramphenicol (80.95%) | Ampicillin (100%) Cefazolin (100%) Erythromycin (100%) |

In this study, all of the GPCs were found sensitive to vancomycin clindamycin susceptibility being observed in 69.23% isolates only while resistance was noted most commonly for erythromycin (88.46%) followed by teicoplanin (84.61%) and penicillin G (84.61%). (Aletayeb et al., 2011) have documented all of their GPCs showing 100% resistance to commonly used antibiotics such as ampicillin and penicillin. GPCs were detected most sensitive to gentamicin, amikacin and tobramycin in a study carried out by (Tallur et al., 2000) while ampicillin was reported as the most resistant antibiotic. (Kaistha et al., 2009) found all their GPCs sensitive to vancomycin (100%), whereas gentamicin (50%) and erythromycin (46.5%) as most resistant antibiotics among all GPCs. (Aletayeb et al., 2011) also documented all of their GPC isolates sensitive to vancomycin (100%) in their study. The reasons for 100% sensitivity of GPCs for vancomycin could be due to its restricted use to
MRSA infections only and also because it was reported resistant by disc diffusion technique which was applied earlier to test vancomycin sensitivity which had further minimized its use in clinical situations by the practicing clinicians. Additionally, it is also one of the rarely used drug like clindamycin and amikacin, hence, these drugs have not developed significant resistance mechanisms unlike any other widely used antibiotics Ampicillin, penicillin and erythromycin have developed high level resistance as they are most commonly and widely used drugs. None of our Gram positive cocci isolates demonstrated 100% sensitivity to any of the antibiotics except vancomycin. Similarly, none of our Gram positive cocci isolates were noted 100% resistance to any of the antibiotics tested – the findings that are noteworthy (Tables 3 and 4).

It was observed that in the present study most of the GNBs were sensitive to imipenem (80.95%), ceftazidime/clavulanic acid (80.95%) and chloramphenicol (80.95%). It is worth knowing and alarming that all our GNBs demonstrated 100% resistance to ampicillin, cefazolin, and erythromycin. (Aletayeb et al., 2011) also reported the 100% resistance of ampicillin and gentamicin to all GNB isolates with 100% sensitive to imipenem. (Tallur et al., 2000) found gentamicin, amikacin, tobramycin as most effective antibiotics and ampicillin as most resistant antibiotic against GNBs. (Kaistha et al., 2009) recorded amikacin and imipenem as most sensitive and effective antibiotics against GNBs whereas amoxicillin (62%) and ampicillin (83%) as least clinically useful antibiotics. Wide variation in antimicrobial sensitivity patterns is noted from region to region and from time to time in the medical literature, which could be to the rise in resistant strains as a result of irrational use of antibiotics in a given defined region.

Most effective antibacterial agents found were vancomycin and clindamycin against GPCs, while imipenem and ceftazidime/clavulanic acid and chloramphenicol against GNBs.

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