The Bacterial Profile and Antibiogram of Neonatal Septicaemia in a Tertiary Care Hospital

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Research Article

Abstract: **Background**: Neonatal septicaemia is a clinical syndrome of bacteraemia characterized by systemic signs and symptoms in first month of life. The type and pattern of organisms that cause neonatal sepsis changes over time and vary from one hospital to another hospital, even in the same country. Early diagnosis and proper management of neonatal septicaemia can bring down the morbidity and mortality substantially. **Objectives: 1)** to isolate and identify the bacterial etiologic agents responsible for neonatal sepsis. 2) To compare and contrast the prevalent bacterial pathogens isolated from early onset neonatal sepsis (EOS) and late onset neonatal sepsis (LOS). 3) To access risk factors associated with neonatal sepsis. 4) To determine the susceptibility pattern of isolates to the commonly used antimicrobial agents in the treatment of sepsis. **Methodology:** Blood culture reports were studied in 306 cases of clinically suspected septicemia in neonates using the standard technique of Mackie and McCartney. The antibiotic sensitivity was performed by Kirby-Bauer's disc diffusion method. Risk factors for sepsis in the children as well as mothers were registered. **Results:** Blood culture reports were positive in 82.35% of cases. Thirty three babies (79%) had early onset sepsis and 9 (21%) had late onset sepsis. *Klebsiella pneumoniae* and *CNS* were the commonest organisms causing neonatal sepsis in both EOS and LOS. Prematurity, lbw and respiratory distress syndrome were strongly associated with blood culture proven neonatal sepsis. Maternal risk factors identified were preterm labour, PROM and intrapartum fever. **Conclusion:** The spectrum of organisms that cause neonatal sepsis changes over time and varies from region to region. These organisms have also developed increasing multi-drug resistance over the last two decades. Therefore knowledge of the pattern of bacterial isolates and their antimicrobial susceptibility pattern is useful for prompt treatment of patients.

Key words: Antibiotic resistance, Neonatal septicaemia.

Introduction

Neonatal sepsis is defined as an invasive bacterial infection which occurs in the first 4 weeks of life. It is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia. The incidence of neonatal sepsis varies from 11-24.5 /1000 live births in India [1]. Sepsis occurring in the first 72 hours of life is defined as early-onset sepsis (EOS) and that occurring beyond 72 hours as late-onset sepsis (LOS). Knowledge of both the common pathogens causing septicemia in neonates and their antimicrobial susceptibility is essential in order to select appropriate antimicrobial treatment. Antimicrobial susceptibility patterns of pathogens vary geographically and are temporally dependent on local pathogens and patterns of antibiotic use. Neonates are more prone to infections due to their weak immunity. Moreover, other risk factors, both in the neonates and in the mothers, are responsible for causing susceptibility to infection. Blood stream infection has been quoted as the most common infection in this age group. In the developing world, *E. coli, Klebsiella species*, and *S. aureus* are the most common pathogens of EOS, whereas *S.aureus*, *Streptococcus pneumonia*, and *Streptococcus pyogenes* are the most commonly reported organisms in LOS. According to the National Neonatal perinatal Database of India, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *E. coli* are the three most common organisms causing neonatal sepsis both in hospital and community. Moreover, the causative organisms of EOS and LOS sepsis are similar especially in hospital setting in developing countries [2]. Septicaemia is a common cause of morbidity and mortality in neonates and children. Numerous risk factors have been identified both in the neonates and children that make them susceptible to infections, which points to the need for bacteriological monitoring in the paediatric wards. The varying microbiological pattern of septicaemia in children warrants the need for an ongoing review of the causative organisms and their antimicrobial susceptibility pattern [3].

Materials and Methods

A cross-sectional prospective study was conducted at the Department of Microbiology, SIMS and Mc Gann hospital, Shimoga from January to December 2012. About 1 ml of blood sample taken from every suspected case of sepsis on admission, before the patient is started on antibiotic therapy, was sent to the Microbiology laboratory. Under proper aseptic conditions, 1ml of venous blood was inoculated in 10 ml of sterile 1% Glucose broth. This was further incubated for 24 hours at

35-37 degree celsius. Blind subcultures were done on 5% Sheep Blood Agar, chocolate and MacConkey's agar after 24 hours, 48 hours, 72 hours, 5th day and final on 7th day as per standard protocol. The blood and MacConkey agar plates were incubated in aerobic and chocolate agar in microaerophilic atmosphere using a candle jar at 37°C for 24-48 hrs [4]. All positive blood cultures were identified by their characteristic appearance on their respective media, gram staining reaction and was confirmed by the pattern of biochemical reactions using the standard method (Cheesbrough, 2001). Members of the family enterobacteriaceae were identified by indole production, H2S production, citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests and other relevant tests. For gram-positive bacteria coagulase, catalase, bacitracin and optochin susceptibility tests, and other tests were done. The negative cultures were sub-cultured at intervals of 48, 72 hours upto 7 days, by standard method [5]. Anti-microbial susceptibility test was performed by modified Kirby Bauer disk diffusion technique using Muller Hinton agar and the results were interpreted according to the CLSI, 2009. From a pure culture 3-5 selected colonies of bacteria was taken and transferred to a tube containing 5 ml sterile normal saline and mixed gently to a homogenous suspension and incubated at 37^{o}C until the turbidity of the suspension become adjusted to a McFarland 0.5. A sterile cotton swab was taken and the excess suspension removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller Hinton agar. The inoculated plates were then left at room temperature to dry for 3-5 minutes and antibiotic discs were placed on the surface of a Muller-Hinton plate. The control strains of E. *Coli* ATCC 25922, *Staph aureus* ATCC 25923 and *Pseudomonas Spp* ATCC 27853 were used. For determining sensitivity, following anti-microbial discs were used. Ampicillin(A) 10µgm, Amikacin(AK) 30µgm, Ceftazidime(CA)30 µgm, Amoxicillin-Clavulanic Acid (AMC) 30 µgm, Oxacillin((OX) 1 µgm, Penicillin(P) 10 units, Vancomycin(VA) 30µg, Cefotaxime CE) 30 µgm, Ceftriaxone(CI) 30µgm, Gatifloxacin(GF) 5µgm, Gentamicin(G) 10 µgm, Erythromycin(E) 15 µgm, Ciprofloxacin(CF) 10µgm and Imepenam(I) 10µgm. Penicillin, Vancomycin, Erythromycin and Oxacillin were tested only for Gram-positive bacteria. The plates were then incubated at 37^{o}C for 24hrs. Diameters of the zone of inhibition around the disc was measured to the nearest millimeter using an digital caliper, and the isolates were classified as sensitive, intermediate, and resistant according to the standard table supplied by the CLSI [5] The newborns included in this study were divided into the following groups:

Group A *Early* **onset sepsis:** including neonates presenting with sepsis before 72 hours of life

Group B *Late* **onset sepsis:** including neonates presenting with sepsis after 72 hours

Observations and Results

A total of 306 cases of clinically suspected neonatal sepsis were included in this study. Among them 252 (82.35%) were culture positive and 54 (17.65%) were culture negative. Among the culture positive a male preponderance (54.7%) was seen. One ninety eight babies (79%) had early-onset sepsis and 54 babies (21%) had late-onset sepsis.

Table 1: Results of blood culture in Neonatal Septicemia

Case reported	No. (n=306)	Percentage
Total no. of positive growth	252	82.35%
Total no. of no growth	54	17.65%

Table 2: Organisms isolated from cases of Neonatal septicaemia

Bacteria	No of cases(n=252)	Percentage
K.pneumoniae	96	38.09
CNS	72	28.57
Citrobacter spp	36	14.28
Acinetobacter	18	7.14
P. aeruginosa	12	4.76
E.coli	12	4.76
S. aureus	6	2.38

In EOS as well as LOS, *K.pneumoniae and Coagulase negative Staphylococcus (CNS)* were the predominant isolates. Over all, Gram negative bacteria formed the major part of isolates at 64.2%. Enterobacteriaceae formed the most prevalent group at 54.7%

Table 3: Organisms isolated from cases of EOS

Bacteria	EOS (n=198)	Percentage
K.pneumoniae	72	36.36%
CNS	42	21.21%
Citrobacter spp	36	18.18%
Acinetobacter	18	9.09%
P. aeruginosa	12	6.06%
E.coli	12	6.06%
S. aureus	6	3.03%

Table 4: Organisms isolated in LOS

Bacteria	LOS(n=54)	Percentage
K.pneumoniae	36	66.66%
CNS	18	33.33%

Table 5: Maternal risk factors

Maternal causes	No. of cases	Percentage
Preterm Labour	54	21.42
PROM	36	14.28
Intrapartum Fever	6	2.38

Table 7: Neonatal risk factors

Neonatal risk factors	No. of cases	Percentage
Preterm	54	21.42
LBW and IUGR	42	16.66
RDS	36	14.28

MAS	30	11.9
Fever	24	9.52
Refused feed	24	9.52
Asphyxia	24	9.52

Antibiotic sensitivity pattern showed that the most commonly isolated organism *Klebsiella pneumonia*e was highly sensitive to gatifloxacin, imipenem, 3rd generation cephalosporins and amikacin. Resistant to gentamycin, ampicillin and amoxy-clav. 50% of the isolates were sensitive to ciprofloxacin. Most *Citrobacter spp* were sensitive to ciprofloxacin, imipenem, gatifloxacin and cefatoxime and resistant to other 3rd generation cephalosporins, aminoglycosides and amoxy-clav. For *Acinectobacter spp* and *P. aeruginosa* imipenem, amikacin, cefatoxime, ceftriaxone were highly sensitive least effective was gatifloxacin, amikacin, ceftazidime, amoxy-clav and gentamycin. *E.coli* were usually sensitive to ciprofloxacin, imipenem, gatifloxacin, amikacin and ceftoxime. Resistant to gentamycin, ampicillin, amoxy-clav and ceftazidime. In gram positive organisms *CNS* was better susceptible to ciprofloxacin, gatifloxacin, amikacin and vancomycin when compared to imipenem and oxacillin. It was resistant to penicillin, erythromycin, cephalosporin and gentamycin. Six *S. auerus* was isolated in our study which were all sensitive to methicillin, ciprofloxacin, imipenem, amoxy-clav and vancomycin, where as resistant to other drugs

Discussion

About five million neonatal deaths occur worldwide every year, 98% of which occur in developing countries, particularly Asia and Africa. Neonatal sepsis generally refers to systemic symptomatic bacterial, fungal, and viral infections that, on earliest presentation, may be associated with any gradation of symptoms, from subtle feeding disturbances to frank septic shock. It is a life threatening emergency and any delay in treatment may result is to frank septic shock [6[. The spectrum of organisms that cause neonatal sepsis changes over time and varies from region to region [7]. These organisms have also developed increasing multi-drug resistance over the last two decades. Therefore knowledge of the pattern of bacterial isolates and their antimicrobial susceptibility pattern is useful for prompt treatment of patients. The present study was undertaken to highlight the pattern of bacterial isolates in neonates and their antimicrobial sensitivity in a tertiary care hospital from January to December 2012. An attempt was also made to identify the possible risk factors responsible for neonatal septicemia. In the present study, out of 306 clinically suspected cases 252 (82%) yielded growth. No growth was seen in 54 (18%) cases. This is high compared to 51% by Karthikeyan [7] and 64% by Tallur [8] et al. We found that early onset sepsis (78.5%) was more common than late onset sepsis (21.5%) which is in agreement with the reports from other developing countries e.g.: in Iran (77.5% vs. 22.5%) [7] and Bangladesh [9] but in contrast with Pakistan [10] (42.% vs. 58%) and Libiya [11] (31% vs. 69%) where LOS in more common. A male preponderance was found in our study which agrees with other studies [5,8,12]. In most of the studies, gram negative bacteria were the principal pathogens causing septicaemia [3, 4, 11] which is similar to our study (64.2%) Enterobacteriaceae formed the most prevalent group at 54.7%. Of the bacterial isolates the most frequent offender was *Klebsiella pneumoniae* (38.09%) followed by *CNS* (28.57%), *Citrobacter spp* (14.28%), *Acinectobacter* (7.14%), *Pseudomonas aeruginosa* (4.76%), *E.coli* (4.76%) and *S.aureus* (2.38%). This preponderance of *Klebseilla pneumoniae* correlates with other studies [5, 8, 13]. In case of gram positive isolates *CNS* were the most common comparable with study of Fleer A et al [14]. In our study *Klebsiella pneumoniae* was the common organism in EOS which is similar to other studies from developing world [8, 13, 15] followed by CNS. We obtained 21.21% of *CNS* in EOS which is similar to Movahedian [7] et al who obtained 20.7% CNS. In contrast in developed nations group *B Streptococci (*GBS) causes upto 52% of EOS [16,17]. GBS is not common in our country and we also did not isolate this organism from our cases. *Klebsiella pneumonia was* also the predominant organism causing LOS followed by CNS. In developed countries, CNS in the major etiological agent for LOS [18, 19]. In our study six isolates of *S. aureus* were isolated all of which were methicillin sensitive. We also obtained *Citrobacter spp* in 18.8% of EOS which was more compared to study done by Ramesh Bhat et al [20]. It was not isolated at all in some studies [8,21]. The isolation rates of *P. aeruginosa and Acinectobacter* were similar to other studies [4, 7]. Studies from different countries report *CNS* as the predominant organisms in LOS [22, 23]. However in this study CNS was recorded from 33.33% cases. Most of the cases detected by blood culture occurred in the first week of life. This calls for close monitoring of the newborns especially those in high risk categories as soon as they are born. Study of maternal risk factors revealed that 54 (21.42%) mothers had preterm labour, 36 (14.28%) had PROM and 6 (2.38%) had intrapartum fever. In this study preterm neonates with LBW and respiratory distress syndrome were at high risk in developing culture proven neonatal sepsis. Neonatal septicaemia is a life threatening emergency and rapid treatment with antibiotics is essential for a favourable outcome. Classical initial (empiric) treatment of neonatal sepsis consists of a combination of penicillin (benzyl penicillin or cloxacillin) and aminoglycosides [24] (most commonly gentamicin)

with the advent of third generation cephalosporins, however, the empiric antimicrobial approach for neonatal sepsis has changed in many centres. Despite this, antibiotic resistance is increasing worldwide particularly in developing countries such as gentamicin resistant *klebsiella spp,* third-generation cephalosporin-resistant gram negative organism, methicillin resistant *Staphylococcus aureus*, vancomicin resistant *Enterococci a*nd penicillin resistant *Streptococcus pneumonia*. Infection with resistant organisms has been associated with treatment failure, higher morbidity and mortality and increased costs. Therefore knowledge of antimicrobial susceptibility pattern of common pathogen of neonatal sepsis in a given area helps to inform the choice of antibiotics. The antibiotics which were used were based on the standard protocol of the hospital and the department policies, which are changed regularly pending the blood culture reports and infections committee recommendations Our results have demonstrated that in general gram negative bacteria isolated from blood culture showed low resistance rates to gatifloxacin, imipenem, amikacin, 3rd generation cephalosporins like ceftazidime and ceftriaxone and gram positive organisms to ciprofloxacin, gatifloxacin, amikacin and vancomicin. Gram negative bacteria showed high level resistance to ampicillin, gentamicin, amoxicillin, clavulinic acid. This observation is comparable to that of other researchers [7, 11]. The major gram positive isolate CNS and S.aureus were frequently found to be penicillin resistant. None of our strains showed resistance against vancomicin and therefore this drug can be effectively used if methicillin resistence is suspected during treatment. Most of CNS were also sensitive to ciprofloxacin, gatifloxacin, amikacin. Maximum resistance was seen to 3rd generation cephalosporins, gentamicin and erythromycin. In the present study gatifloxacin was the most effective drug against *Klebsiella pnuemoniae*, *Citrobacter* and *E.coli* whereas imipenem was the most effective against *P.aeruginosa* and *Acinectobacter.* Other drugs to which they were sensitive was ceftriaxone, ceftoxime and amikacin. No difference was seen in the antibiogram of the organism causing EOS and LOS Generally it is not an easy task to compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable. Few studies compare antibiotic susceptibility over time in the same unit, but where data are available they show increasing resistance to commonly used antibiotics.

Conclusion

Klebsiella pneumoniae and *CNS* were the commonest organisms causing neonatal sepsis in both EOS and LOS. Prematurity, lbw and respiratory distress syndrome were strongly associated with blood culture proven neonatal sepsis. Maternal risk factors identified were preterm labour, PROM and intrapartum fever. Gram negative bacteria showed high level resistance to commonly used drugs like ampicillin, gatifloxacin, amoxy-clav, gentamicin, imipenem, amikacin and 3rd generation cephalosporins like ceftazidine and ceftriaxone were most effective for gram negative organism. The gram positive organisms were penicillin resistant (100%) and CNS was resistant to methicillin in 66% cases. Empirical antibiotic regimen for gram positive and gram negative sepsis must take into consideration the high rates of ampicillin and gentamicin resistance that are now prevalent. The antibiotic sensitivity profile suggests that gatifloxacin and 3rd generation cephalosporins are most suitable drugs for treatment of gram negative neonatal septicaemia. Routine bacterial surveillance and the study of their resistance patterns must be an essential component of neonatal care. Knowledge of these patterns is essential when local policies on the uses of antibiotics are being devised. Recovery of CNS from blood of a septicaemia neonate needs to be reviewed with caution since not all of them are true bacteraemia agents. The role of anaerobic bacteria, group B streptococci and fungi in neonatal sepsis could not be investigated in this study. Investigation of co-existing infections by other clinical samples in addition to blood is recommended which was was not done in this study.

Acknowledgments

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