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To Study Bacteriological Profile and Antibiotic Sensitivity Pattern in Cases of Neonatal Sepsis

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Abstract

Objective: To study the bacteriological profile and sensitivity pattern in cases of Neonatal sepsis at 12 bedded Neonatal Intensive Care Unit (NICU). **Methods:** This was a prospective study of neonates admitted to our NICU from Jan 2010 to Oct 2011 with diagnosis of neonatal sepsis or those who developed sepsis later during their stay in NICU. All newborns diagnosed as a case of neonatal sepsis, based on clinical features with positive sepsis screen and/or positive blood culture, were included in our study. Blood Culture & Sensitivity was done with conventional non-automated method using Herley's Broth. **Results:** Out of 55 cases blood culture was positive in 27 (49.09%) cases. Klebsiella Pneumoniae 15 (55.55%) was the most common organism isolated in both early and late onset sepsis showed sensitivity to collistin in 86.6% of cases followed by sensitivity to imipenam-cilastin in 46.67% cases. Only 20% Klebsiella isolates were sensitive to drugs like Ampicillin-sulbactum, Amikacin, Tazobactum, cefpime and for other antibiotics like Meropenam, Piperacillin-tazobactum, Vancomycin it was less than 20%. Other organisms isolated were Staph. aureus, E. Coli, Pseudomonas, Enterobactor, Acinetobactor and candida species. **Conclusion:** Gram negative organisms are most common cause of early as well as late onset sepsis and there is alarming degree of antibiotic resistance to commonly used antibiotics.

Keywords: Antibiotic Sensitivity, Bacteriological Profile, Gram Negative Organisms, Neonatal Sepsis

1. Introduction

Neonatal sepsis is responsible for about 30-50% of the total neonatal deaths in developing countries[1]. Infection claims the lives of approximately 3000 live neonates worldwide every day[2]. In India as per national Neonatal Perinatal Database (NNPD 2002-2003), the incidence of neonatal sepsis was 30 per thousand live births and it was found to be one of the commonest causes of neonatal mortality contributing to 19 % of all noenatal deaths[3]. It has also stated that among the intramural babies the incidence of neonatal sepsis was 3.0% which accounted for 16% of total deaths in intramural babies while among the extramural babies incidence of neonatal sepsis was 39.7% which accounted for 38% of total deaths in extramural babies[4]. The sepsis rate in preterm infants is considerably higher at 54.4 per 1000 preterm live births[5].

In developed world, more than half of the neonates admitted to NICU carry a diagnosis of 'rule out sepsis' and these infants account for up to 25% of NICU days

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in some units[6]. Neonatal sepsis is defined as a clinical syndrome of bacteraemia characterised by systemic signs & symptoms of infection in first 28 days of life. It encompasses various systemic infections of newborn such as septicaemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection[7]. Neonatal sepsis can be classified into two sub-types depending upon whether the onset of symptoms is before 72 hours of life (early onset) or later (late onset). This classification is based on the fact that early-onset infections are more likely to be caused by organisms prevalent in the maternal genital tract or in the delivery area. Early onset sepsis manifests frequently as pneumonia and less commonly as septicaemia or meningitis[3]. Late-onset sepsis is caused by the organisms thriving in the external environment of the home or the hospital. The infection is often transmitted through the hands of the care-providers. The onset of symptoms is usually delayed beyond 72 hours after birth and the presentation is that of septicaemia, pneumonia or meningitis. The associated factors of late-onset sepsis include: low birth weight, lack of breastfeeding, superficial infections (pyoderma, umbilical sepsis), aspiration of feeds, disruption of skin integrity with needle pricks and use of intravenous fluids[3]. Sepsis related mortality is largely preventable with early & rational antimicrobial therapy and aggressive supportive care. However delay in treatment has catastrophic results. Although positive blood culture is the gold standard for diagnosis of Neonatal sepsis, the results are available only after 48 to 72 hrs so investigators have devised sepsis markers (sepsis screens) for early detection of neonatal sepsis to enable the clinician to start treatment before the blood culture results are available.

The problem of neonatal sepsis is also complicated by its changing bacteriological profile and sensitivity pattern of the organisms showing resistance to increasing number of antibiotics. Thus a rational protocol for sepsis management must be based on continuously updated knowledge of the prevalent organisms and their sensitivity pattern. The present study is undertaken to address these very issues.

2. Material and Methods

All newborns diagnosed as a case of neonatal sepsis, based on clinical features with positive sepsis screen and/or positive blood culture, were included in our study. This was a prospective study of neonates admitted to our NICU from Jan 2010 to Oct 2011 with diagnosis of neonatal sepsis or those who developed sepsis later on during their stay in NICU. A detailed history including the history of risk factors (PROM, foul smelling/meconium stained liquor, maternal fever, more than 3 PV examinations, prolonged labour with instrumentation, perinatal asphyxia) & examination of the baby was recorded on a printed proforma Samples were then collected for CBC, BSL, CRP & Blood culture and sensitivity in all cases while other investigations like CSF, urine, stool (occult blood) and imaging studies such as X-ray chest, abdomen, USG, NSG were done as per the case. Methodology of CBC, CRP, Blood Culture - CBC – Sample was collected in the EDTA bulb. CBC was checked manually under microscope after staining and with cell counter (ERMA or BECKMAN COUNTER) in the pathology lab. CRP was done using "RHELAX-CRP slide test". It is based on the principle of agglutination. The test specimen (serum) is mixed with RHELAX-CRP latex reagent and allowed to react. If CRP concentration is greater than 0.6mg/dl a visible agglutination was observed. If the CRP concentration is less than 0.6mg/dl then no agglutination is observed. Sepsis screen was taken as positive if two or more than two of the following parameters were abnormal[8] – 1. C Reactive protein - >0.6 mg/dl, 2. Total leukocyte count - <5000/cmm^3, 3. Absolute Neutrophil count - <1000/cmm^3, 4. Immature to total neutrophil ratio - >0.2, 5. Degnerative changes of leukocyte - Present/Absent. Blood Culture & Sensitivity was done with conventional non-automated method using Herley's Broth.

3. Results

A total of 55 patients were enrolled in the study from JAN 2010 to Aug 2011. Out of these 55 cases, 15 cases were in tramural and 40 cases were extramural. 45(82%) were preterm and 10(18%) were term. The gestational age of the study sample ranged from 25 to 40 weeks with a mean of 32.04 wks, 33% had a gestational age range from 28-30wks & 31% had the gestational age from 31-33wks. 35(63.6%) were Male and 20(36.4%) were Female, showing a preponderance of male babies but the difference was not statistically significant (p>0.05). Among the study cases the weight ranged from 851gms to 3kgs with a mean of 1591.6gms. Maximum no of the patients belonged to the 1001 to 1500 gm group. The majority of the cases were appropriate for gestational age. A majority (N=39) were delivered by the vaginal route. 46 babies had positive sepsis screen (sensitivity 84%), while 27 babies had a positive blood culture (sensitivity 49.09%). Out of the 55 babies, 28 (50.90%) babies had a positive sepsis screen but their blood culture was negative. Both blood culture

and sepsis screen were positive in 18 (32.72%) cases while in 9 babies (16.36%) only blood culture was positive. *Klebsiella* was isolated in total 15 cases among which, in 5 cases another organism was also isolated in same blood culture. It included the combinations of either *E. coli* and *Klebsiella* (1case), *Candida* & *Klebsiella* (1 case), *Acinetobactor* and *Klebsiella* (one case) & *Staph aureus* & *Klebsiella* (2 cases). The frequency of organisms is shown in Table 1.

Table 1. A total of 32 organisms were isolated from 27 blood cultures, in 5 cases 2 organism each were isolated on blood culture

Organism isolated	*N (%)*
Klebsiella	*15 (55.55%)*
Staph Aureus	*5 (18.51%)*
E-coli	*4 (14.18%)*
Pseudomonas	*3 (11.11%)*
Enterobactor	*2 (7.40%)*
Acinetobactor	*2 (7.40%)*
Candida	*1 (3.70%)*

Blood culture was found to be positive more often in LOS (59.26%) than EOS (37%) but the difference was not statistically significant (p>0.05). Gram negative organisms were the most common pathogen both in EOS and LOS. Gram positive organisms were mainly isolated in LOS and were the second most common organisms. Among the gram negative organisms, *Klebsiella* was the commonest isolate in both EOS (43.75%) & LOS (22.58%). Among the EOS cases the next common organism found were pseudomonas and *E. coli* after *Klebsiella* while among the LOS cases, *Staph. aureus* was the major causative agent after *Klebsiella*. After applying Chi square test there is no significant association between organisms isolated in blood culture and age of onset of sepsis (0>0.05). In the study group of 55 cases, the CSF examination was done in 6 cases & in 2 cases CSF findings were suggestive of bacterial meningitis. One patient had a positive blood culture in which growth of *Klebsiella* and *Staph. aureus* was present. Three out of 55 cases had conjunctivitis with purulent eye discharge but only one culture showed growth of *Acinetobacter*. In two cases the endotracheal tube culture sensitivity showed growth of *Klebsiella* and *Pseudomonas*. One of our cases had right hip joint swelling which was aspirated and sent for culture and sensitivity which showed the growth of *Candida albicans* but the blood culture was negative. Two patients had abscess formation at the IV sites. The pus from these showed growth of *Klebsiella* and *Acinetobacter* respectively.

3.1 Antibiotic Sensitivity

Klebsiella which was the commonest of all isolated, organisms showed sensitivity to colistin in 86.6% of cases followed by its sensitivity to imipenam (46.67%). Only 20% of *Klebsiella* isolates were sensitive to drugs like ampicillin-sulbactum, amikacin, tazobactum, cefepime, sparfloxacin while for the other antibiotics eg. meropenam, piperacillin-tazobactum, vancomycin the sensitivity was less than 20%. *Staph. aureus* showed sensitivity to linezolid, amikacin, vancomycin, imipenam, colistin in 40-80% of cases while in only 20% cases it was sensitive to ampicillin-sulbactum, cefotaxim, ceftriaxone, amoxy-clavulanic acid & ofloxacin. Third most common organism isolated in our study was *E. coli* and it again showed low degree sensitivity (25% each) of sensitivity to common antibiotics such as ampicillin-sulbactum, amikacin, ceftazidime, cefoperazone-sulbactum and netlimicin. In 75% of cases *E. coli* showed sensitivity to only 2 antibiotics - colistin and imipenam. Pseudomonas was sensitive to colistin, piperacillin-tazobactum (100% in all cases) followed by ofloxacin & amoxicillin-clavulinic acid (66.66% cases each). In small percentage of cases (33.33%) it was sensitive to ceftazidime, imipenam, ampicillin-sulbactum, amikacin. Enterococcus showed sensitivity to ofloxacin in both the cases while it showed sensitivity to colistin, imipenam, meropenam, vancomycin, linezolid in one case only. Acinetobector showed 100% sensitivity to colistin, ampicillin-sulbactum & cephotaxim. The *Candida albicans* which was isolated in one case showed sensitivity to Amphoterecin B and Nystatin.

3.1.1 Antibiotic Sensitivity Pattern of Organisms Isolated from Other Sites

Endotracheal tube C/S: In two cases the Endotracheal tube culture sensitivity showed growth of *Klebsiella* and *Pseudomonas*. *Klebsiella* was sensitive only to Colistin and Imipenam. Psudomonas sensitive to Colistin Cephoperazone-sulbactum and Gatifloxacin only. Pus culture: In the one case the pus culture showed growth of Klebsiella and *Acinetobector* both of them were sensitive to antibiotic Cefepime, Colistin and tazobactum only. In the 2nd case the pus culture showed growth of *E. coli* which was sensitive to ceftazidime, colistin and ampicillin-sulbactum. Eye discharge: In one of the three patients with eye discharge the culture showed growth of Acineobactor which was sensitive to ampicillin-sulbactum, cephoperazone-sulbactum,

colistin, piperacillin-tazobactum, ofloxacin and Resistant to amikacin, cephotaxim, imipenam, netilimycin.

4. Discussion

The bacteriological profile of neonatal sepsis varies according to the region of the world as well as according to the time of onset of sepsis. The spectrum of pathogens in south Asia and Africa is quite different from that observed in neonates in developed regions. The microorganisms most commonly reported from developed world to be associated with early onset infection include group B *Streptococcus* (40.7%) and *E. coli* (17.2%); others being *Strepto. viridans, Entercoccus* and *Staph. aureus*.[10].

In developing countries gram negative pathogens are the predominant causative organisms for early onset sepsis mainly represented by *Klebsiella, E. coli*, and *Pseudomonas*. Of the gram positive organisms *Staph. aureus*, CoNS, *Streptococcus pneumoniae* and *Streptococcus pyogenes* are common isolates. Group B *Streptococcus* is generally rare or not seen at all[11]. Almost half of neonatal bloodstream infections in developing regions are due to gram-negative pathogens such as *Klebsiella, Pseudomonas* and *Acinetobacter* spp. that cause common-source outbreaks because they thrive in multi-use containers of medications, liquid soaps and other solutions, including antiseptics and disinfectants and on inadequately reprocessed equipment[12]. *Klebsiella pneumoniae* is the major pathogen, responsible for 16–28% of blood-culture confirmed sepsis in different regions of the world[12].

K. pneumoniae needs to be recognised as one of the most important neonatal pathogens in developing countries. Incidence of neonatal *Klebsiella* infection varies between 4.1 and 6.3 per 1000 live births with case fatality rates of 18–68% .The rate of *Klebsiella* infections in the Indian National Neonatal - Perinatal Database dataset was 5.7 per 1000 live births, two to four times higher than the rate of neonatal group B streptococcal infections in the industrialized world[12].

Under certain circumstances fungal infections also play an important role in complicating neonatal infections. *Candida* and *Malassezia* species are the two most frequent groups of opportunistic organisms causing disseminated fungal infection in premature neonates under 1500 g. In our study Blood culture was positive in 49.09% cases. Blood culture positivity in our study is comparable to that of Roy et al. & Tallur et al.[13]. However Joshi et al. found blood culture to be positive only in 25% of his cases[14]. The wide differences in this regard may be due to factors such as time of sampling, extent of bacteremia in neonate and prior antibiotic treatment in the neonate. In our study group, out of the 27 culture positive cases 22 (81.48%) showed growth of gram negative organism while 3 (11.11%) showed growth of gram positive organism. Two blood cultures showed growth of both gram positive and negative organisms. This is quite similar to the results of the other workers (Table 2).

Table 2. Comparative table showing the distribution of gram positive and gram negative organisms

GRAM Positive/ Negative Organism.	Khatua et al. (18) n=92 (1986)	Sharma et al. (68) n=521 (2002)	Tallur et al. (21) n=203 (2003)	Present study n=55 (2010)
Gram +ve	23.7%	15%	18%	11.11%
Gram –ve	76.3%	85%	82%	81.48%
Both Gram +ve & Gram –ve	_	_	_	7.40%

4.1 Organsums Isolated

In our study group of 55 cases of neonatal sepsis, *Klebsiella* was the most common organism isolated both in EOS and LOS groups. *Klebsiella* as the commonest pathogen for neonatal sepsis has also been documented by Tallur et al.[13] and NNPD[4] Database 2002-2003. However *E. coli* was reported as the most common pathogen by Kuruvilla et al.[15] *S. Aureus* was the second most common organism isolated (all cases among LOS group) and *E. coli* was the third most common organism (14.18%) responsible for neonatal sepsis. We did not find any case of Group B Streptococcus (GBS) sepsis. This is in sharp contrast to the trend in the Western developed countries where it is a major agent of early onset neonatal septicemia[16]. In our study in one case of LOS, *Candida Albicans* was isolated. This baby was on antibiotics for more than three weeks.

4.2 Antibiotic Sentivity/Resistance Pattern among the Organisms Isolated

Only 20% of *Klebsiella* isolates were sensitive to drugs like ampicillin-sulbactum, amikacin, tazobactum, cefepime, sparfloxacin while for the other antibiotics eg.

meropenam, piperacillin-tazobactum, vancomycin the sensitivity was less than 20%. The sensitivity pattern of *Klebsiella* to Ampicillin & amikacin is quite comparable to that of of NNPD database 2002-2003.

S. aureus which was the 2nd most common organism in our study showed sensitivity to ampicillin-sulbactum, cloxacillin, ceftriaxone, cefepime, ofloxacin & clindamycin in only 20% of cases while the maximum sensitivity of 80% was found against linezolid, amikacin followed by vancomycin (60%). In 75% cases *E. coli* was sensitive only to colistin & imipenam. However in the NNPD database *E. coli* showed relatively more sensitive to common antibiotics eg. amikacin (76.7%), ciprofloxacin (45.2%), ceftazidime (51.5%), cephotaxim (45.2%). However, it was sensitive to ampicillin in only (14.1%) cases. Tallur et al.[13] in his series of cases found that. *E. coli* was sensitive to ampicillin in only 14.1% cases, it retained its sensitivity to other antibiotics e.g. cefotaxim (100%), ciprefolxacin (100%) & amikacin (86%).

Pseudomonas in our series showed 100% sensitivity only to colistin and piperacillin-tazobactum, while it was sensitive to ampicillin-sulbactum, cefotaxim, ceftazidime & imipenam in only 33.33% of the cases. Enterococcus in our study, showed sensitivity of 50% only to amikacin, ceftazidime, cefotaxim imipenam, clindamycin.

Acinetobactor in our series showed maximum sensitivity to ampicillin-sulbactum, cephotaxim & colistin (100%). To other antibiotics such as imipenam, amoxy-clavulanic acid, cephalexin, piperacillin tazobactum, ofloxacin, cloxacillin, ceftriaxone, sparflox and lomefloxacin it showed sensitivity in 50% cases only.

In one case in our study *Candida albicans* was isolated which was resistant to all the routine antifungal drugs such as fluconazole, variconazole, meconazole & ketoconazole but it was sensitive to Amphoterecin B and Nystatin. After taking into account all the available database it is apparent that both the *Klebsiella* and *Staph. aureus* together have contributed to nearly half of all cases of neonatal sepsis and both these organisms have developed significant resistance to nearly all commonly used antibiotics[17]. *Klebsiella* has developed resistance to all commonly used penicillins and cephalosporins. Ampicillin resistance is seen in nearly 85% of isolates[17] Gram negative organism predominated in both early & late onset sepsis. *Klebsiella* was the most common organism isolated in both EOS & LOS. Mortality was seen in 29.1% of cases while 61.80% cases survived. Mortality was higher among males as compared to females but the difference was not statistically significant. In our study group, we found maximum mortality (50 to 100%) in patients who showed a combination of both gram positive & gram negative organisms and a combination of gram negative organism with *Candida*. Patients with pure gram negative sepsis had a much lower mortality (21%) while there was no mortality in those with gram positive sepsis alone. Mortality rate was highest in cases of pseudomonas sepsis & in those with mixed sepsis 66.66% each, followed by *E. coli* (25%), *Klebsiella* (11.11%). Similar findings have been noted by many other workers in the field[14,18,19]. It is apparent from the antibiogram that almost all the organisms isolated in blood culture except for *S. aureus* (80% sensitive to Amicacin) and acinetobactor showed sensitivity in only 20-33% of patients to the first line antibiotics that were used in our NICU such as ampicillin-sulbactum and amikacin. In the majority of patients the organisms showed sensitivity only to a few reserve drugs like Colistin, Imipenam etc. In the light of the limitations imposed by the sensitivity pattern of the common pathogens of our NICU, it is suggested that the third generation cephalosporin and amikacin combination should be the first line of antibiotics. Imipenam & linezolid combination should be the second line of antibiotics while colistin & vanciomycin should be kept as reserve drugs.

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