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Bacteriological Profile and Antibiotic Sensitivity Pattern of Neonatal Sepsis in a Tertiary Care Hospital

Pavneet Kaur* and Sarbjeet Sharma

Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, India

**Corresponding author*

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A B S T R A C T

India accounts for 30% of neonatal deaths globally. Bacterial sepsis is a significant cause of morbidity and mortality in newborns. The study helps to make antibiotic policy in neonatal sepsis. The main objective is to study the incidence of multidrug resistant gram negative and gram positive organisms causing neonatal septicemia and their antibiotic sensitivity pattern. The study was conducted in the Department of Microbiology over a period of one year. Sample of blood was collected under aseptic precautions and processed by standard techniques. Microorganisms were identified by Gram staining, standard biochemical tests and appropriate antibiograms. The common microorganisms responsible for neonatal sepsis were identified, and the resistant strains were studied. After identification and antibiotic susceptibility testing, beta-lactamases were detected as per CLSI guidelines. In 233 blood cultures 18.9 % (44/233) culture positivity was seen. Out of them, 31 (70.5%) were Gram negative and 13(29.5%) were Gram positive. *Klebsiella pneumoniae* subspecies *pneumoniae* (45.5%) was the most common isolate. ESBL producers were maximum (54.8%.) 25.8% of the isolates were positive for AmpC production. The diverse microbiological pattern of neonatal septicaemia demands the need for review of neonatal sepsis. The evaluation of the pathogens and their antibiotic susceptibility is a relevant guide in the antibiotic therapy.

Introduction

Neonates with low immunity, always need prolonged hospitalization which is a risk factor of post-infectious complications. (Adams-Chapman and Stoll, 2002) It is estimated that about 5 million neonatal deaths occur in a year out of which 98% occur in developing countries. (WHO, 1996) Medical advancements of the last twenty years have increased the survival rate of neonates. Most common causative bacteria in developed countries includes Coagulase-negative staphylococcus and group B Streptococcus while in developing countries are *E. coli, Klebsiella, Enterobacter* etc. (Waheed *et al.,* 2003) *Klebsiella* and *Enterobacter* species are often associated with the production of extended-spectrum beta-lactamase (ESBL) among the multiresistant Gram-negative bacteria. (Karunasekera and Pathirana, 1999) The control of these hospital acquired infections has been a challenging task. (Goldmann *et al.,* 1983) Spectrum of

multiresistant organisms causing neonatal sepsis is variable over time and across countries. Thus periodic surveillance is essential.

Materials and Methods

A prospective study was conducted over a period of one year in the department of Microbiology in a tertiary care centre. Blood was collected from 233 cases of neonatal septicemia from NICU, under aseptic precautions and processed by standard techniques. Standard microbiological techniques were used for identification the organisms grown in pure culture (Collee *et al.*, 1996). Antibiotic susceptibility testing of Gram-negative isolates was done on Mueller-Hinton agar plates by the Clinical and Laboratory Standards Institute (CLSI)-recommended Kirby-Bauer disc diffusion method with discs of Gentamicin (10 µg), Amikacin (30 µg), third-generation Cephalosporin (30 µg), Ciprofloxacin (5 µg), Piperacillin-Tazobactam (100 + 10 µg) and Meropenem (10 µg). (Clinical and laboratory Standard Institute Guidelines, 2013; Lalitha, 2011) The isolates were tested for ESBL production using double disc approximation test and CLSI phenotypic confirmatory test. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the negative and positive controls. Amp-C β-lactamases producers were screened by using reduced cefoxitin susceptibility and production was confirmed by modified three dimensional test. An indentation or a flattening of the zone of inhibition indicated AmpC production (Shahid *et al.*, 2004).

Antibiotic sensitivity tests of staphylococcal isolates were done in Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method. Antibiotics used were Gentamicin (10 µg), Erythromycin (15 µg), Cefoxitin (30 µg), Amikacin (30 µg), Rifampicin (5 µg), Clindamycin (2 µg), Linezolid (30 µg). The control strain used was *S. aureus* ATCC 25923. The results were interpreted according to the CLSI guidelines (Clinical and laboratory Standard Institute Guidelines, 2013; Lalitha, 2011)

Results and Discussion

Out of 233 newborns with neonatal sepsis included in the study, 44 (18.9%) positivity was seen. The predominant organisms responsible for septicemia were *S. aureus, Klebsiella Enterobacter, Pseudomonas* and *Acinetobacter* spp. Among the 44 isolates, 31 (70.5%) were Gram-negative organisms and 13 (29.5%) were Gram-positive organisms.

Klebsiella pneumoniae subspecies pneumoniae was the most common organism isolated 20 (45.5%) followed by *Staphylococcus aureus* 12(27.3), *E. coli* 7 (15.9%), *Acinetobacter* 2(4.5%) and *Pseudomonas* 2 (4.5%). Table 1 and 2 show the antibiotic sensitivity pattern of Gram-negative and Gram-positive isolates, respectively. The total prevalence of ESBL production in this study was 54.8%. Among *K. pneumoniae*, 60% were ESBL producers whereas 40% were non-ESBL. 4(57.1%) out of 7 *E. coli* were ESBL producers. Among the 31 Gram-negative isolates, 8 (25.8%) were AmpC production positive.

Among Coagulase positive staphylococci, all were methicillin sensitive (MSSA). The sensitivity of amikacin and ciprofloxacin showed sensitive trend with most of the gram negative organisms. Imepenem was maximum sensitive among all gram negative organisms. Third generation with inhibitor i.e. Pipra + tazobactam also showed good sensitivity as shown in Table 1. In case of Gram positive Vancomycin, clindamycin, cefoxitin and Linezolid, were found most effective as shown in Table 2.

Neonatal sepsis is an invasive infection usually bacterial / fungal in origin and documented by a positive blood culture. It is the most common constituent contributing to NICU admissions in developing countries. Blood culture positivity in these cases has shown wide variation over the years. (Mondal *et al.*, 1991) It is a leading cause of morbidity and mortality.

In the present study, blood culture was positive in 18.9 %. The predominant organisms isolated were *Klebsiella* spp and *S. aureus* which is similar to a study from (NNPD, 2000). Thus, *Klebsiella* spp and *S.aureus* are the most common causative organisms responsible for neonatal septicemia in India.

Of the 44 culture positive isolates, 20 (45.5%) were of *Klebsiella* spp. The most sensitive drugs were Imipenem, Piperacillin + tazobactam and Cefoperzone +sulbactam combination.

Table.1 Sensitivity and resistance percentage of gram negative organisms to antibiotics

Antibiotic	E coli (7)		*Klebsiella* (20)		*Pseudomonas* (2)		*Acinetobacter* (2)	
	S %	R%	S%	R%	S%	R%	S%	R%
Gentamicin	42.9	57.1	30	70	50	50	50	50
Amikacin	85.7	14.3	40	60	50	50	50	50
Ciprofloxacin	71.4	28.6	60	40	100	0	50	50
Ceftriaxone	42.9	57.1	35	65	NT*	NT*	50	50
Imepenem	100	0	100	0	100	0	100	0
Pipra +Tazo	85.7	14.3	85	15	100	0	50	50
Cefoperazone + sulbactum	71.4	28.6	85	15	NT*	NT*	50.0	50.0

*NT=Not tested

Table.2 Sensitivity and resistance percentage of gram positive organisms to antibiotics

Antibiotic	S aureus(12)		CONS(1)	
	S %	R %	S %	R %
Cephalexin	100	0	100	0
Gentamicin	75	25	100	0
Amikacin	100	0	100	0
Cefoxitin	100	0	100	0
Vancomycin	100	0	NT*	NT*
Erythromycin	66.7	33.3	100	0
Rifampicin	100	0	100	0
Clindamycin	100	0	100	0
Linezolid	100	0	NT*	NT*

*NT=Not tested

The present study outlines the importance of higher generation cephalosporin with beta lactamases inhibitor combination for empirical therapy.

In a study by Shah *et al.,* in 2012, Gram-negative organisms were isolated in 52% cases, Gram-positive in 45% cases and Candida species in 3% cases. (Shah *et al.,* 2012) The increased incidence of sepsis by Gram-negative bacteria may be attributed to the fact that there is colonisation of Gram-negative bacteria in the skin of the neonate and the personnel of the neonatal wards.

In a study by (Abdel-Hady *et al.,* 2008) 67% of the *Klebsiella* isolates were ESBL producers which is consistent with the present study.

ESBLs production is not routinely tested in most centres resulting in the dissemination of ESBL-producing strains within and between hospitals and remains undetected for long periods. Similar to study conducted by (Jain and Mondal, 2007), a high incidence of lactamases production in *Klebsiella* spp was observed, which may be due to injudicious use of antibiotics. Hence, prophylactic use of antibiotics should be with caution, while therapeutic antibiotics should be specific and used for as short a period of time as possible. Rotating antibiotic regimens is suggested in conditions where the use of antibiotics is necessary.

There was no MRSA among gram positive isolates in this study. 33 % of CONS were found in the present study which can be decreased by following appropriate aseptic precautions in collection of blood samples. Relevant educational programs combined with rotational antibiotic therapy may be useful in fighting against such types of infections. Considering widespread antibiotic resistance in our country, it is essential to determine the prevalence of β-lactamases producers to aid in formulating an antibiotic policy for empirical therapy in NICU.

The varying microbiological pattern of neonatal septicaemia warrants the need for periodic review of neonatal sepsis as the knowledge of the pathogens and their antibiotic susceptibility would be a useful guide in the antibiotic therapy This will also facilitate infection control interventions like hand hygiene, patient isolation and contact precautions

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