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Bacteriological profile of neonatal sepsis and pattern of antimicrobial susceptibility in a tertiary care hospital of Amritsar

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ABSTRACT

Objective: To study the bacteriological profile of neonatal sepsis and pattern of antimicrobial susceptibility in a tertiary care hospital of Amritsar from January 2013 to June 2014.

Methods: The study was done on all the neonates admitted with clinical signs of septicemia. Blood samples were collected using aseptic precautions and subjected to bacteriological culture and antimicrobial susceptibility testing using standard established techniques. Results: Of the total 512 clinically suspected cases of sepsis, bacterial agents were isolated from 232 (45.3%) cases. Percentage of Gram positive organisms was 33.2% and of Gram negative organisms was 66.8%. Most common isolate was Klebsiella pneumonia followed by Coagulase Negative Staphylococci (CONS). The most common isolate in Early Onset Sepsis (EOS) was CONS and in Late Onset Sepsis (LOS) Klebsiella pneumonia. Gram positive organisms were most sensitive to linezolid and amikacin whereas Gram negative organisms showed maximum sensitivity to imipenem and combination of ceftizidime-sulbactam. Risk factors associated with neonatal sepsis were low birth weight (64.2%), preterm birth (43.7%), premature rupture of membranes (PROM) (23%), home delivery (19.2%) and maternal fever (17.3%).

Conclusion: Multidrug resistant Gram negative isolates are frequently encountered in cases of neonatal sepsis. Good patient care practices and rational use of antibiotics can go a long way to reduce infection rate in neonates and to ensure better therapeutic success.

KEYWORDS : Bacteriological profile, Neonatal sepsis, Antimicrobial susceptibility

Introduction

Neonatal sepsis is a significant cause of morbidity and mortality among neonates worldwide. It is responsible for 30-50% of total neonatal deaths in developing countries[1]. Neonatal sepsis may be defined as clinical syndrome characterized by signs and symptoms of infections with or without accompanying bacteremia in first month of life. It is classified as Early Onset Sepsis (EOS) and Late Onset Sepsis (LOS) [2]. EOS occurs within first 72 hours of life[3] and is acquired in utero or during intrapartum period. LOS occurs after 72 hours of life. It may be nosocomial or community acquired[4].

There are various reasons and conditions which make the neonatal period highly vulnerable for infections. The most common risk factor associated with EOS include maternal Group B Streptococcus colonization, maternal fever, maternal urinary tract infection, prolonged rupture of membranes, Prematurity, chorioamnionitis, foul smelling/meconium stained liquor amnii, poor prenatal care, low Apgar score (<6 at 1 or 5 min), low birth weight, prolonged and difficult delivery with instrumentation, birth asphyxia and congenital anomalies[5,6]. Late onset sepsis is generally associated with NICU admission, prematurity, low birth weight, poor hygiene, poor cord care, bottle-feeding and prelacteal feeds, invasive procedures, superficial infections, continuous positive airway pressure (CPAP) use, H2 blocker/proton pump inhibitor use and gastrointestinal tract pathology[7]. Bacterial agents implicated in EOS includes *Group B streptococcus, Escherichia coli, CONS, Haemophilis influenzae* and *Listeria monocytogenes*[8, 9]. The organisms commonly associated with LOS include *CONS, Staphylococcus aureus*, *Klebsiella spp, Escherichia coli, Enterobacter spp, Pseudomonas aeruginosa* and *Acinetobacter spp.* Early diagnosis and treatment goes a long way in reducing morbidity and mortality but over the last few decades development of resistance in causative agents to commonly used antibiotics has made management of the patients a major problem.

The present study was designed to evaluate the common pathogens associated with neonatal septicemia in our hospital and their antibiotic susceptibility pattern over a period of 18 months (January 2011 to December, 2013).

Material and Methods

The present study was conducted in Microbiology department of Government Medical College & associated GND hospital, Amritsar. The study population was all neonates clinically diagnosed as cases of neonatal sepsis & admitted in pediatrics ward from January 2013 to June 2014. Blood samples were collected in Brain Heart infusion broth using aseptic techniques. The bottles were shaken gently and incubated at 37^{0}C. After overnight incubation the broth was subcultured on MacConkey, blood and chocolate agar. Any growth was identified by colony characters, gram staining and various established biochemical tests. Negative results were followed up by examining the broth daily and doing a final subculture on 7th day before reporting it as negative. The isolates were tested for their antimicrobial susceptibility by Kirby-Bauer disc[10] diffusion method as recommended by Clinical Laboratory Standard Institute (CSLI) [11]. Commercially available antibiotics discs (Hi-media, Mumbai) were used for susceptibility testing. The following antimicrobials were tested for gram positive organisms - Ampicillin- 10ug/disc, Amikacin- 30ug/disc, Gentamycin- 10ug/disc, Ceftrixone- 30ug/disc, Cephalexin-30ug/disc, Ciprofloxacin- 5ug/disc, Erythomycin- 15ug/disc, Linezolid- 10ug/disc and Cefoxitin-30ug/disc.

For Gram negative organisms antibiotics tested were - Amikacin- 30ug/disc, Gentamycin- 10ug/disc, Ciprofloxacin- 5ug/disc, Ceftrixone -30ug/disc, Ceftizidime- 10ug/disc, Ceftizidime-Sulbactum- 10/30ug/disc, Piperacillin-Tazobactam- 100/10ug/disc and Imipenem - 10ug/disc.

Statistical analysis[12]. The results were analysed using the statistical package for Social science (SPSS)/ 16.0 (Copyright © SPSS Inc.).

Results

Among the 512 neonates admitted with suspected sepsis, blood cultures were positive in 232 (45.3%) cases. Gram negative bacteria were isolated in 155 (66.8%) cases whereas Gram positive isolates in

77 (33.1%) cases. The most common causative agent was *Klebsiella pneumoniae* 58 (25%) followed by *CONS* 47 (20%), *Acinetobacter spp* 32 (13.8%), *Staphylococcus aureus* 27 (11.6%), *Citrobacter spp* 24 (10.7%), *Escherichia coli* 22 (9.5%), *Pseudomonas spp* 15 (6.4%), & *Enterococcus spp* 3 (1.3%).

In EOS, Gram positive isolates were more common. *CONS* were the commonest 33 (35.4%) followed by *Klebsiella* pneumoniae 21 (22.5%), *Escherichia coli* 17 (18.2%) and *Staphylococcus aureus* 17(18.2). (Table 1)

In LOS, Gram negative isolates were more common. *Klebsiella pneunoniae* was the commonest isolate 37 (26.6%) followed by *Acinetobacter spp* 32 (23%), *Citrobacter spp* 21 (15.1%), *Pseudomonas spp* 14 (10.0%) and *Staphylococcus aureus* 10 (7.2%).

In our study, >60% Gram positive isolates showed resistance to ampicillin, gentamicin, ciprofloxacin, cephalexin and ceftriaxone which are commonly used in our neonatal ICU however amikacin, linezolid and cefoxitin showed sensitivity in >80% isolates. In general the most effective drug against the Gram-negative bacteria was Imipenem (95.4%) followed by ceftizidime-sulbactum (74.2%) and least effective drugs were ceftizidime followed by ceftriaxone and ciprofloxacin.

Table 1 Bacterial etiological agents isolated from blood culture in neonates with suspected sepsis

Etiological agents	EOS No (%)	LOS No (%)	TOTAL No (%)
Klebsiella pneumoniae	21 (22.5)	37 (26.6)	58 (25)
CONS	33 (35.4)	14 (10.0)	47 (20.2)
Acinetobactor spp	0 (0)	32 (23.0)	32 (13.8)
Staph aureus	17 (18.2)	10 (7.2)	27 (11.6)
Citrobacter spp	3 (3.2)	21 (15.1)	24 (10.7)
Escherichia coli	17 (18.2)	5 (3.6)	22 (9.5)
Pseudomonas spp	1 (1.0)	14 (10.0)	15 (6.4)
Enterococcus spp	1 (1.0)	2 (1.4)	3 (1.3)
Others	0 (0)	4 (2.8)	4 (1.7)
Total	93 (40.0)	139 (60.0)	232 (45.3)

EOS: Early-onset sepsis LOS: Late-onset sepsis

More than 60% of *Klebsiella pneumoniae* isolates were multi drug resistant i.e. resistant to amikacin, gentamicin, ciprofloxacin, ceftiazidime and piperacillin-tazobactum. The data was given to incharge NICU to formulate new antibiotic policy.

In our study, neonatal factors significantly associated (p<0.001) with culture proven sepsis were gestational age < 37 weeks and low birth weight. Out of 224 preterm neonates, 140 (62.5%) were culture positive and out of 329 neonates with birth weight <2500 gm, 188 (57.1%) were culture positive.

Table 2 Association of various neonatal and maternal risk factors with culture positive sepsis

Factors	Value	Culture positive No (%)	Culture negative No (%)	Total	P value
Gestational Age	<37 Week (preterm)	140(62.5)	84(37.5)	224	<0.001
	37-42 weeks (term)	92(33.0)	186 (70.0)	278	
	>42weeks (post term)	0 (0)	10(100)	10	
Weight at birth (BW)	<1500g (VLBW)	56 (58.3)	41 (42.7)	96	< 0.001
	<2500g (LBW)	132 (56.6)	102 (43.7)	233	
	2500-4000g	44 (24.8)	133(75.1)	177	
	>4000g (overweight)	0 (0)	4 (100)	4	
Place of delivery	Hospital /Inborn	123 (40)	192 (60.0)	315	0.002
	Health centre	80 (57.1)	62 (43.9)	142	
	Clinic	19 (47.5)	21 (52.5)	40	
	Home	10 (66.6)	5 (33.3)	15	
Mode of delivery	Vaginal (NVD)	193 (52.5)	175 (47.6)	367	< 0.001
	Caesarian (LSCS)	32 (23.8)	102 (76.1)	134	
	Instrumental(IVD)	7 (63.6)	4 (36.3)	11	
PROM>24 hours	Yes	74 (60.0)	56 (40.0)	132	0.002
	No	158 (41.4)	224 (58.6)	382	
Maternal fever	Yes	32 (72.7)	12 (27.3)	44	<0.001
	No	200 (42.7)	268 (57.3)	468	
Parity	Primiparous	107 (46.5)	123 (53.5)	230	0.620
	Multiparous	125 (44.3)	157 (55.7)	282	
Foul smelling liquor	Yes	22 (57.9)	16 (42.1)	38	0.105
	No	210 (44.3)	264 (55.7)	474	

[p > 0.05- Not Significant, p<0.05- Significant, p<0.001- Highly significant]

Most important maternal risk factors which were significantly associated with culture proven sepsis were PROM >24 hours (60%) (P<0.005) and history of maternal fever (72.7%) (p<0.001) (Table2). Other factors associated with culture positive sepsis were home delivery and instrumental vaginal delivery. Percentage of culture positive sepsis in home delivered neonates was 66.6% and in neonates with instrumental vaginal delivery 63.6%.

Discussion

Sepsis is still a major cause of mortality and morbidity in the first month of life. New treatment alternatives are being explored throughout the world because of its changing bacterial profile and high mortality rate. Gram-negative organisms continue to be a menace to the sick, fragile and debilitated newborns. In our study *Klebsiella pneumoniae* was the most common isolate 58 (25%) causing neonatal sepsis followed by *CONS* 47 (20.1%) and *Acinetobacter spp* 32 (13.8%). Similar spectrum of bacterial isolates was seen in study by Zakariya et al who also reported *Klebsiella pneumonia* as the most common (66%) cause of neonatal sepsis followed by *CONS* (12%) and *Acineobacter spp* (6%).[13] In the present study, the most common isolates in EOS were *CONS* (35.4%) followed by *Klebsiella pneumonia* (22.5%). Similar results were given by Gheibi et al who also reported *CONS* as commonest (48.8%) cause of EOS followed by *Klebsiella pneumonia, E.coli* and *Staphylococcus aureus*.[14] whereas Zakariya et al in their study reported *Klebsiella pneumonia* as the commonest (74.4%) isolate in EOS and *CONS* were the second common isolate[13]. In the current study, the most common pathogen isolated in LOS was *Klebsiella pneumonia* (26.6%) followed by *Acinetobacter spp* (23.0%). Gram negative isolates (81.3%) were significantly more common (p<0.001) than Gram positive isolates (18.7%). Zakariya et al also reported *Klebsiella pneumonia* (36.4%) as most common causative agent of LOS followed by *CONS* (23.7%) and *Acinetobacter spp* (9.1%).[13] In contrast a study done by Roy I et al in a tertiary care hospital of North India, *Enterobacter spp* (23.3%) was the major pathogen in LOS followed by *CONS* (22.3%).[15]

The susceptibility pattern of Gram positive and Gram-negative organisms to the most relevant antibiotics is depicted in table 2. In our study Gram positive isolates showed high level of resistance (>80%) to Ampicillin (82%) and Cephalexin (89.7%), intermediate level resist-

ance (60-80%) to Ceftriaxone (75.3%), Ciprofloxacin (74%) and Erythromycin (69.0%). Most effective drug in Gram positive isolates were Linezolid (96.1%), Cefoxitin (93.5%) and Amikacin (80.0%) for *Staphylococcus aureus* and *CONS* and Vancomycin for *Enterococcus spp.* Vancomycin sensitivity was tested only for *Enterococcus spp*. Similarly Roy et al reported more than 89% Gram positive isolates were resistant to Ampicillin and >40% were resistant to Erythromycin, Gentamicin and Ciprofloxacin.[15] Amikacin resistance was infrequent and none of the Gram positive isolate were resistance to Vancomycin.[15] In our study Gram negative bacteria showed high-level resistance (>80%) to Ceftizidime and Ceftriaxone, Intermediate level resistance (60-80%) to Ciprofloxacin and Gentamicin. In general the most effective drugs against Gram negative isolates were Imipenem (95.4%) followed by Ceftazidime-Sulbactam (74.2%) and Amikacin (54.2%) The most common isolate in our study, the *Klebsiella pneumoniae* was found to be highly resistant showing multi drug resistance to Ciprofloxacin (84.4%), Ceftizidime (81.0%), Gentamicin (70%), Piperacillin-Tazobactum (63.8%) and Amikacin (55.1%). Most effective drug in thses cases was Imipenem (96.5%) followed by Ceftizidime-Sulbactam (70.0%). Similar results were reported by Zakariya B P et al in which *Klebsiella pneumonia* was resistant to most drugs except Amikacin and Meropenem.[13] The detection of multi-drug resistant isolates may further limit therapeutic options and necessitating the role of culture and sensitivity.

Table 3 Antimicrobial susceptibility pattern of bacterial isolates

Antimicrobial	Gram positive isolates No (%)	Gram Negative Isolates No (%)
Ampicillin	14 (18.1)	84(54.2)
Amikacin	61 (80.0)	60 (38.7)
Gentamicin	40 (52.0)	46 (40.0)
Ciprofloxacin	20 (26.0)	46 (40.0)
Cephalexin	8 (10.3)	-
Ceftriaxone	19(24.7)	36 (23.2)
Ceftizidime	-	32 (20.6)
Erythromycin	24 (31.1)	-
Imipenem	-	147 (95.4)
Linezolid	74 (96.1)	-
Cefoxitin	72 (93.5)	-
Piperacillin-Tazobactam	-	73 (47.1)
Cetizidime-Sulbactam	-	115 (74.2)

In this study low gestational age and low birth weight were significantly associated ($p<0.001$) with culture proven neonatal sepsis (table 3). Culture positivity rate was high (62.5%) in preterm neonates (<37 weeks) and neonates with low birth weight (<2500gm) (57.1%).

Most important maternal risk factors showing statistically significant association with culture positive sepsis in our study were PROM>24 hours ($p<0.005$) and history of maternal fever ($p<0.001$). Alam et al also reported significant association of PROM, maternal fever and chorioamnionitis with culture proven sepsis.[16]

Home delivery ($p<0.005$) and instrumental vaginal delivery ($p<0.001$) were other important factors showing statistically significant association with culture proven sepsis in this current study.

Conclusion

In our study, Gram negative organisms were the predominant cause of neonatal sepsis more so in LOS. *Klebsiella pneumoniae* was the most common isolate, followed by CONS and *Acinetobacter spp*. The organisms were resistant to most of the commonly used antibiotics. Multi-drug resistance was detected in >60% *Klebsiella pneumonia*. Prematurity, low birth weight, PROM >24 hours, history of maternal fever and instrumental vaginal delivery were identifiable risk factors for culture proven sepsis. Therefore, the management strategy for these high-risk neonates in developing countries should focus on identification of risk factors, recognition of clinical condition with prompt laboratory screening for sepsis and early institution of empirical antibiotic treatment. The empirical regimen should be modified from time to time based on the antibiograms of the isolates.

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