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Original Article

Microbiology Section

Causative Agents, Antibiotic Resistance Patterns and Risk Factors in Early and Late Onset Neonatal Sepsis in a Neonatal Intensive Care Unit

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ABSTRACT

Introduction: Neonatal sepsis is one of the major causes of neonatal mortality. Causative agents, antibiotic resistance patterns and risk factors of sepsis differ from one region to another in the world. Periodic assessments of these will guide to use an appropriate antibiotics for rational empirical treatment and contribute to management of sepsis.

Aim: To determine causative agents, antibiotic resistance patterns and risk factors of early and late onset neonatal sepsis.

Materials and Methods: A total of 230 neonates diagnosed as sepsis were included in this retrospective study. Data collection was made by reviewing records of patients from laboratory and hospital information system of Konya Dr Ali Kemal Belviranlı Gynaecology, Obstetrics and Children Hospital between September 2015-May 2017. Blood samples were incubated in automated blood culture system. Subcultures were performed on 5% sheep blood agar and eosin methylene blue agar. Identification of microorganisms was firstly made by conventional methods. Further identification and antibiotic susceptibility tests were performed by automatic analyser. Results were considered statistically significant at $p<0.05$.

Results: Early Onset Sepsis (EOS) was detected in 19 (8.3%) neonates while Late Onset Sepsis (LOS) occured in 211 (91.7%) of them. Coagulase-negative *Staphylococcus* was the most commonly isolated microorganisms in both early and late onset sepsis with rates of 84.2% and 81.5%, respectively. The rate of gram negative microorganisms was 13% and Enterobacteriaceae compose 8.7% of these. *Klebsiella pneumoniae* (3.5%) was the most common bacteria within Enterobacteriaceae. High methicillin resistance rates (81.2% and 83.7%) were observed in Coagulase negative *Staphylococcus* in both EOS and LOS, respectively. Enterobacteriaceae had extended spectrum beta-lactamase positivity rate of 63.2% and showed high resistance to ceftriaxone (63.2%) and cefuroxime (78.9%). No carbapenem resistance were detected in gram negative bacteria. No significant associations was found between onset of sepsis and gestational age, gender and mode of delivery ($p>0.05$).

Conclusion: There were some differences and also similarities between aetiologic agents and antibiotic resistance patterns among data obtained in this study and those from other countries. This study contributed to epidemiological information and routine antibiotic surveillance to use appropriate antibiotics for empirical treatment of sepsis and prevent its complications.

Keywords: Aetiology, Bacterial profile, Blood cultures, Empirical treatment, Septicaemia

INTRODUCTION

Sepsis is one of the most common cause of neonatal mortality worldwide [1,2]. World Health Organisation (WHO) predicts approximately 5 million neonatal deaths a year. The majority of these deaths were attributed to sepsis and reported from developing countries [3-6]. Neonatal sepsis, a clinical condition emerging in the first 28 days of life, is characterised by systemic symptoms and isolation of microorganisms from bloodstream [4,7,8]. It is classified as early and late according to time of onset. EOS is a confirmed infection in blood during the first 72 hours of life. LOS occurs after the first three days of life. EOS is usually related to intrapartum transmission from maternal genital tract while LOS is caused by postnatal acquisition of microorganisms from hospital [7,9,10].

Determination of causative agents of EOS and LOS is important for prevention and treatment [4]. Microorganisms causing neonatal sepsis and their response to antibiotics vary with geographical areas and level of development of countries. While Group B *Streptococcus* is the most common cause of EOS in developed countries, Enterobacteriaceae is main cause in

developing countries [4,8,10,11]. The most commonly isolated microorganisms in LOS are *Staphylococcus* species and Gram-negative bacteria such as *Klebsiella* species and *Escherichia coli*. Antibiotic resistance rates of microorganisms causing LOS are higher than those of EOS. The major risk factors for LOS are low birth weight, use of intravascular catheters, endotracheal intubation, mechanical ventilation, contact with contaminated equipment and hand of hospital staff [7,9,11].

Blood cultures and antibiotic susceptibility tests are important for managing appropriate antibiotic treatment in neonatal sepsis. However, these tests give results between 48-72 hours after the samples are obtained and therefore initially empirical antibiotic treatment is applied for the most likely pathogen bacteria which are thought to be susceptible to chosen antibiotics [5,11]. Antibiotic resistance of microorganisms causing neonatal sepsis decreases the effectivity of empirical treatment and also makes the treatment choices limited [6]. The aim of this study was to determine causative agents, antibiotic resistance patterns and associated risk factors among neonates with EOS and LOS and to guide empirical antibiotic treatment in Neonatal Intensive Care Units (NICUs).

MATERIALS AND METHODS

A total of 230 neonates diagnosed as proven sepsis with positive blood cultures and hospitalised in Neonatal Intensive Care Unit (NICU) of Konya Dr Ali Kemal Belviranlı Gynaecology, Obstetrics and Children Hospital between September 2015-May 2017 for a period of 20 months were included in this retrospective study. Records of patients were reviewed retrospectively from laboratory and hospital information system digitally for the following variables: gender, age, birth weight, gestational age, mode of delivery, diagnosis, invasive intervention, maternal risk factors, isolated microorganisms and antibiotic resistance paterns. Written informed consent was obtained from the parents of neonates.

Ethical Consideration

The study was approved by Review Board and Ethics Committee of Konya Necmettin Erbakan University Meram Faculty of Medicine (Ref No:2018/1434).

Inclusion Criteria

Neonates with proven sepsis at the time of admission or who developed sepsis during their stay in hospital were enrolled in this study. Blood cultures were obtained from at least two separate sites of neonates by applying optimal antiseptic technique. Coagulase-negative *Staphylococcus* (CoNS) which were accepted as aetiologic agents of sepsis were isolated from two or more blood cultures obtained at one time within the same day. If the same bacteria isolated from at least two positive blood cultures on the same day or consecutive days in neonates with evidence of sepsis based on clinical and laboratory findings, these bacteria were reported as aetiologic agents of sepsis.

Exclusion Criteria

Neonates who had already received antibiotic treatment before obtaining blood cultures were excluded from this study. Microorganisms like CoNS, which were isolated from only one blood culture of a neonate were accepted as contaminants.

Sample Collection and Laboratory Procedures

Using aseptic technique, 1-4 mL of blood samples for blood cultures were taken after peripheral venipuncture from each arm and inoculated directly into blood culture bottles (BacT/ALERT PF Pediatric FAN) containing tryptone soy broth. Samples were processed on arrival to the laboratory and blood culture bottles were incubated in BacT/ALERT 3D (bioMérieux, France) automated blood culture system for five days. Aerobic conditions were provided by the analyser and incubator. When the analyser detected growth, blood culture bottles yielded positive signal. Gram stain was done from these positive signaling bottles and subcultures were performed by inoculating directly on 5% sheep blood agar and eosin methylene blue agar. Eosin methylene blue agar was used for isolation of gram negative microorganisms as it is a selective agar medium. Identification of gram positive and gram negative organisms were done by conventional methods. These methods were colony morphology on agar mediums, haemolytic activity on 5% sheep blood agar, gram stain appearance, catalase and coagulase reaction, (for gram positive cocci) and other standard commercially prepared biochemical tests. Further identification and antibiotic susceptibility tests were performed by VITEK® analyser (bioMérieux, France). Antibiotic susceptibility of all isolates were determined by breakpoints for Minimum Inhibitory Concentrations (MIC) according to the criteria established by European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12]. Results were quantitative for MIC values and qualitative expressed as susceptible, intermediate or resistant. Vancomycin and teicoplanin resistances was confirmed by antibiotic gradient tests (Bioanalyse, Turkey). Colistin and tigecycline MIC values were tested and calculated by automated analyser VITEK® (bioMérieux, France). MIC values, susceptibility and resistance patterns were evaluated according to instructions of EUCAST [12].

STATISTICAL ANALYSIS

Statistical analysis were performed by Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Inc, Chicago, IL, USA). Descriptive analysis were expressed as percentages (%) for categorical variables and mean±Standard Deviation (SD) for quantitative variables. Chi-Square Monte Carlo exact test was used for analysis of relationships between categorical data. The

results were considered statistically significant at probability p-value <0.05.

RESULTS

A total of 230 neonates diagnosed as sepsis with positive blood cultures were included in this study. Among them, 115 (50%) were males and 115 (50%) were females. The mean age was 13.12±7.85 days (2-30 days). The average gestational age was 35.99±3.29 weeks (24-41 weeks). The average birth weight was 2721.87±706.93 gram (600-4600 grams). EOS was detected in 19 (8.3%) neonates while LOS occured in 211 (91.7%) of them. Of the total 230 neonates, 89 (38.7%) were preterm and 141 (61.3%) were term. The rates of preterm neonates in LOS and EOS were 37.4% and 52.6%, respectively. EOS was detected in 52.6% of females and 47.4% of males. The rates of LOS in females and males were 49.8% and 50.2%, respectively. It was recorded that 141 (61.3%) neonates were born by vaginal delivery while 89 (38.7%) of them were delivered by caesarean section. There were no significant associations between onset of sepsis and variables such as gestational age, gender and mode of delivery (p>0.05). Among 211 neonates with LOS, 26 (12.3%) of them had birth weight between 2001-2500 grams and there were 6 (31.6%) neonates with EOS in the same group regarding birth weight. The differences between these rates were found as statistical significant (p<0.05) [Table/Fig-1]. Neonatal pneumonia (27%) was the most frequently reported diagnosis among neonates with sepsis. This was followed by neonatal jaundice (16.5%) and respiratory distress syndrome (15.2%). The rates of EOS and LOS among neonates diagnosed as neonatal pneumonia were 52.6% and 24.6%, respectively. There were statistical significant differences between these rates (p<0.05). Also, differences between the rates of EOS and LOS among neonates with meconium aspiration syndrome were found as statistically significant (p<0.05). Among 230 neonates, 188 (81.8%) of them had invasive intervention and the most performed intervention was mechanical ventilation with the rate of 62.8%. During gestational period, 15 (6.5%) neonates mothers had maternal

	EOS		LOS		Total		p*
	n	%	n	%	n	%	
Gender							0.811
Female	10	52.6	105	49.8	115	50	
Male	9	47.4	106	50.2	115	50	
Gestatıonal age							0.467
<37 (preterm)	10	52.6	79	37.4	89	38.7	
37-42 (term)	9	47.4	132	62.6	141	61.3	
Bırth weıght							0.583
≤1000	0	0	10	4.7	10	4.3	
1001-1500	1	5.3	7	3.3	8	3.5	
1501-2000	2	10.5	14	6.7	16	7	
2001-2500	6	31.6[a]	26	12.3[a]	32	13.9	
>2500	10	52.6	154	73	164	71.3	
Mode of delıvery							0.507
Vaginal delivery	13	68.4	128	60.7	141	61.3	
Caesarean section	6	31.6	83	39.3	89	38.7	

[Table/Fig-1]: Demographic characteristics of neonates with early and late onset neonatal sepsis.
a: Differences shows significant rates, EOS: Early-onset sepsis; LOS: Late-onset sepsis;* Chi-Square monte carlo exact test

	EOS		LOS		Total		p*
	n	%	n	%	n	%	
Clinical diagnosis							0.687
Neonatal pneumonia	10	52.6[a]	52	24.6[a]	62	27	
Neonatal jaundice	1	5.3	37	17,5	38	16.5	
Respiratory distress syndrome	1	5.3	34	16,1	35	15.2	
Transient tachypnea of the neonate	3	15.8	18	8,5	21	9.1	
Metabolic disease	0	0	21	10	21	9.1	
Congenital anomalies	0	0	13	6.2	13	5.7	
Meconium aspiration syndrome	3	15.8[b]	5	2.4	8[b]	3.5	
Bronchiolitis	0	0	8	3.8	8	3.5	
Unable to feed	0	0	7	3.3	7	3	
Urinary tract infection	0	0	5	2.4	5	2.2	
Meningitis	1	5.3	4	1.9	5	2.2	
Omphalitis	0	0	4	1.9	4	1.7	
Hydrocephaly	0	0	3	1.4	3	1.3	
Invasıve intervention							0.288
Mechanical ventilation	15	83.3	103	60.6	118	62.8	
Umbilical venous catheterization	1	5,6	30	17.6	31	16.5	
Orogastric tube	0	0	22	12.9	22	11.7	
CPAP device	1	5,6	12	7.1	13	6.9	
Exchange transfusion	1	5,6	3	1.8	4	2.1	
Maternal risk factors							0.740
PROM	2	66.7	8	66.7	10	66.7	
Urinary tract infection	1	33.3	3	25	4	26.7	.
Diabetes mellitus	0	0	1	8.3	1	6.6	

[Table/Fig-2]: Clinical characteristics of neonates with early and late onset neonatal sepsis.
EOS: Early-onset sepsis; LOS: Late-onset sepsis; CPAP: Continuous positive airway pressure; PROM: Premature rupture of membranes; a,b: Differences shows significant rates; *Chi-Square monte carlo exact test

risk factors and the most seen risk factor was Premature Rupture Of Membranes (PROM) (66.7%). There was not a significant association between onset of sepsis and these variables (p>0.05) [Table/Fig-2].

The majority of blood culture isolates were gram positive bacterial pathogens with the rate of 87%. CoNS were the most common isolated gram positive microorganisms in cases of both EOS and LOS with the rates of 84.2% and 81.5%, respectively. Of the total of 188 (81.7%) CoNS; 78 (41.5%) were *Staphylococcus epidermidis*, 40 (21.3%) were *Staphylococcus hominis*, 37 (19.7%) were *Staphylococcus warneri* and 33 (17.5%) were *Staphylococcus haemolyticus*.

It was detected that 30 (13%) isolates were gram negative bacteria and *Klebsiella pneumoniae* was the most common gram negative organism with 8 (3.5%) isolates. In LOS, *Klebsiella pneumoniae* (3.3%) was followed by *Enterobacter cloacae* complex (2.4%), *Escherichia coli* (1.9%) and *Pseudomonas aeruginosa* (1.9%). Only 1 (5.3%) isolate of Enterobacteriaceae and non-fermenting bacteria (5.3%) was detected in EOS. *Streptococcus* spp. (2.6%) were rarely detected among all 230 neonatal sepsis cases. *Enterococcus spp*. was isolated from only 1 (0.5%) case of LOS [Table/Fig-3]. High resistance rates (81.2% and 83.7%) to methicillin was observed in CoNS in both EOS and LOS, respectively. Methicillin resistance was detected in only one *Staphylococcus aureus* strain isolated from a neonate with LOS. The distribution of methicillin resistance rates for gram positive bacteria was found as statistically significant (p<0.05). No glycopeptide and linezolid resistant *Staphylococcus* species were recorded in EOS and LOS. Only one glycopeptide resistant *Enterococcus faecium* was isolated in LOS. Ciprofloxacin had similar resistance rates for CoNS in both EOS and LOS cases (43.8% and 42.4%, respectively) but erythromycin had resistance rates of over 60% for these bacteria. *Streptococcus agalactiae* strains were sensitive to all antibiotics [Table/Fig-4]. In Enterobacteriaceae, extended spectrum beta-lactamase (ESBL) positivity rate was 63.2% and these bacteria showed high resistance to ceftazidime (63.2%), ceftriaxone (63.2%) and cefuroxime (78.9%). Less resistance was reported to aminoglycosides, mainly amikacin (31.6%) and gentamycin (47.4%). No carbapenem, colistin and tigecycline resistance was detected in Enterobacteriaceae. On the other hand, the resistance rates of carbapenems (both imipenem and meropenem) among non-fermenting bacteria isolated from neonates with LOS were 25%. The resistance rates of ciprofloxacin were less than 25% in both Enterobacteriaceae and non-fermenting bacteria. No levofloxacin resistance was detected [Table/Fig-5].

Microorganism	EOS		LOS		Total		p*
	n	%	n	%	n	%	
***Staphylococcus* spp**							0.497
CoNS	16	84.2	172	81.5	188	81.7	
Staphylococcus aureus	0	0	5	2.4	5	2.2	
Enterobacteriaceae							0.299
Klebsiella pneumoniae	1	5.3	7	3.3	8	3.5	
Enterobacter cloacae complex	0	0	5	2.4	5	2.2	
Escherichia coli	0	0	4	1.9	4	1.8	
Enterobacter aerogenes	0	0	3	1.4	3	1.3	
Non-Fermenting Bacteria							0.880
Pseudomonas aeruginosa	0	0	4	1.9	4	1.8	
Pseudomonas putida	1	5.3	2	0.9	3	1.3	
Pseudomonas mendocina	0	0	1	0.5	1	0.4	
Sphingomonas paucimobilis	0	0	1	0.5	1	0.4	
Stenotrophomonas maltophilia	0	0	1	0.5	1	0.4	
***Streptococcus* spp**							0.480
Streptococcus agalactiae	1	5.3	3	1.4	4	1.8	
Streptococcus pneumoniae	0	0	2	0.9	2	0.8	
Enterococcus spp	0	0	1	0.5	1	0.4	-

[Table/Fig-3]: Distribution of microorganisms isolated from neonates with early and late onset neonatal sepsis.
EOS: Early-onset sepsis; LOS: Late-onset sepsis; CoNS:Coagulase-negative Staphylococci

DISCUSSION

Sepsis is a major cause of prolonged hospital stay, morbidity and mortality in neonates. Microorganisms that cause neonatal sepsis vary from one country to another in the world [5,13]. LOS was more common than EOS in present study with the rate of 91.7%. This rate was higher than the reported rates of LOS as 34.4 % in Iran, 34.5% in India, 48,7% in Indonesia 51.4% in Tanzania, 55.8% in Egypt and 70.8% in Pakistan [3,6,8,14-16]. The differences of LOS rates among countries may be related to variabilities of epidemiology of neonatal sepsis, antibiotic treatment policies and protective measures of neonatal units of hospitals.

Recent studies showed that CoNS are the predominant cause of LOS [17]. Prematurity, low birth weight and interventions like intravascular catheters which remain for prolonged periods are risk factors for CoNS infections [5,18-20]. In present study, CoNS were the most common

	CoNS		*Staphylococcus aureus*		*Streptococcus agalactiae*		*Streptococcus pneumoniae*		*Enterococcus* spp.		p*
	EOS n (%)	LOS n (%)	EOS n (%)	LOS n (%)	EOS n (%)	LOS n (%)	EOS n (%)	LOS n (%)	EOS n (%)	LOS n (%)	
Ciprofloxacin	7 (43.8)	73 (42.4)	-	0 (0)	-	-	-	-	-	0 (0)	0.058
Erythromycin	10 (62.5)	143 (83.6)	-	2 (40)	-	-	-	0 (0)	-	-	0.001*
Clindamycin	7 (43.8)	85 (49.4)	-	1 (20)	0 (0)	0 (0)	-	1 (50)	-	-	0.439
Oxacillin	13 (81.2)	144 (83.7)	-	1 (20)	0 (0)	0 (0)	-	1 (50)	-	-	0.001*
Methicillin	13 (81.2)	144 (83.7)	-	1 (20)	0 (0)	0 (0)	-	1 (50)	-	-	0.001*
Cefoxitin	13 (81.2)	144 (83.7)	-	1 (20)	-	-	-	-	-	-	0.001*
Gentamycin	5 (31.2)	83 (48.3)	-	0 (0)	-	-	-	0 (0)	-		0.034*
Vancomycin	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	-	0 (0)	-	1 (100)	-
Teicoplanin	0 (0)	0 (0	-	0 (0)	0 (0)	0 (0)	-	0 (0)	-	1 (100)	-
Linezolid	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	-	0 (0)	-	0 (0)	-
SXT	4 (25)	41 (23.8)	-	1 (20)	0 (0)	0 (0)	-	-	-	1 (100)	0.579
Tetracycline	10 (62.5)	110 (64)	-	2 (40)	-		-	-	-	-	0.035*
Fosfomycın	6 (37.5)	58 (33.9)	-	3 (60)	-		-	-	-	-	0.228
Levofloxacin	-	-	-	-	-		-	0 (0)	-		-
Tigecycline	0 (0)	0(0)	-	0 (0)	-		-	0 (0)	-	-	-
Penicillin					0 (0)	0 (0)	-	1 (50)	-	-	-

[Table/Fig-4]: Antibiotic resistance patterns of gram positive bacteria isolated from blood cultures.
EOS: Early-onset sepsis; LOS: Late-onset sepsis; CoNS: Coagulase-negative Staphylococci; SXT: Trimethoprim/Sulphamethaxazole; *Chi-Square monte carlo exact test; Differences shows significant rates

	Enterobacteriaceae spp		Non-fermenting bacteria		p*
	EOS n (%)	LOS n (%)	EOS n (%)	LOS n (%)	
Ampicillin	1 (100)	17 (89.5)	-	-	-
AMC	1 (100)	15 (78.9)	-	-	-
Amikacin	0 (0)	6 (31.6)	0 (0)	0 (0)	0.077
Gentamycin	1 (100)	9 (47.4)	0 (0)	2 (25)	0.289
Ceftazidime	1 (100)	12 (63.2)	0 (0)	3 (37.5)	0,229
Ceftriaxone	1 (100)	12 (63.2)	2 (25)	2(25)	-
Cefuroxime	1 (100)	15 (78.9)	-	-	-
Cefepime	1 (100)	9 (47.4)	0 (0)	2 (25)	0.289
Ciprofloxacin	0 (0)	4 (21.1)	0 (0)	1 (12.5)	0.608
Ertapenem	0 (0)	0 (0)	-	-	-
Imipenem	0 (0)	0 (0)	0 (0)	2 (25)	0.202
Meropenem	0 (0)	0 (0)	0 (0)	2 (25)	0.144
SXT	1 (100)	10 (52.6)	-	-	-
Levofloxacin	-	-	0 (0)	0 (0)	-
Piperacillin-tazobactam	1 (100)	5 (26.3)	0 (0)	2 (25)	-
Tigecycline	0 (0)	0 (0)	-	-	-
Colistin	0 (0)	0 (0)	0 (0)	0 (0)	-
Netilmycin	-	-	0 (0)	0 (0)	-
ESBL	1 (100)	12 (63.2)			-

[Table/Fig-5]: Antibiotic resistance patterns of gram negative bacteria isolated from blood cultures.
EOS: Early-onset sepsis; LOS: Late-onset sepsis; AMC: Amoxicillin/Clavulanic acid; SXT: Trimethoprim/Sulphamethaxazole; ESBL: Extended spectrum beta-lactamase

Authors	EOS (%)	LOS (%)
Shehab El-Din EMR et al., [6]	65.3	46.1
Acquah SE et al., [5]	33.3	38.5
Tehrani FHE et al., [14]	37.2	16.1
Present study	84.2	81.5

[Table/Fig-6]: Isolation rates of CoNS in early and late onset sepsis [5,6,14].
EOS: Early-onset sepsis; LOS: Late-onset sepsis

isolated microorganisms in both EOS and LOS with the rates of 84.2% and 81.5%, respectively, which was similar to the previous studies while lower rates were detected in some studies [Table/Fig-6].

On the other hand, some studies reported *Staphylococcus aureus* as the most common microorganism isolated from neonatal sepsis [4,8,9,21,22]. Contrary to this, *Staphylococcus aureus* (2.2%) was rarely isolated in this study.

It was reported that bacteriological profile changed as gram-negative, mostly *Klebsiella* species (33.3%) in neonatal sepsis in a study carried out by Pokhrel B et al., from Nepal [23]. *Klebsiella pneumoniae* was also reported as the most common cause of neonatal sepsis in studies conducted in Egypt, Mexico and India [10,11,15]. Hasibuan BS from Indonesia detected the most common isolated microorganism in neonatal sepsis as *Klebsiella pneumonia* (19.5%), followed by *Acinetobacter baumanii* (16.9%) and *Enterobacter cloacae* (8.4%) [16]. In present study, the isolation rate of *Klebsiella pneumoniae* (3.5%) was lower than these studies. Also, if we have a look at non-fermenting bacteria, *Pseudomonas* spp was isolated from blood cultures with the rate of 3.5%. On the other hand, in a study from India, *Pseudomonas aeruginosa* was the most common encountered microorganism in both EOS (43.8%) and LOS (51.3%) [24]. In the present study, group B streptococci (1.8%) were rarely isolated from neonates with EOS and LOS as the same as studies from Tanzania and Mexico [4,11]. Contrary to these findings, a study from Taiwan reported Group B streptococci as the most common microorganism in EOS [25].

In this study, the rates of EOS and LOS in males and females were similar and there was no statistical significant association between gender and occurence of EOS and LOS like other studies conducted in some countries [3,6,14,26]. The opposite was found in studies from Indonesia, Egypt and Pakistan that neonatal sepsis occured more common in males compared to females [7,10,13,16]. Many studies reported that prematurity is an important risk factor for sepsis [9,14,27]. The rate of preterm neonates with sepsis was 38.7% in the present study and this finding agreed with previous reports [9,10,15]. Ozdemir AA and Elgormus Y from Istanbul, Turkey found a lower prematurity rate of 19.8% [27]. However, a higher rate was reported (68.1%) from a study conducted by Pokhrel B et al., from Nepal [23]. In this study, it was found that there was no significant association between gestational age and onset of sepsis. This finding was in accordance with a report from Tanzania [3]. However, in studies from Mexico and Taiwan, a meaningful association was detected between gestational age less than 37 weeks and onset of sepsis [11,25].

Hayun M et al., from Indonesia demostrated that the risk of EOS in neonates with birth weight <1500 gram was 4.9 times greater than those with birth weight ≥1500 gram [26]. This finding was supported by a study from Gondar, Ethiopia [9]. Some studies indicated that the incidence of sepsis was higher in neonates born via caesarean section than in those born via vaginal delivery [6,9]. In present study, only 10 (4.3%) neonates had very low birth weight (<1000g) and vaginal delivery with the rate of 61.3% was more than caesarean section.

Perinatal factors such as PROM, infection and malnutrition increase the risk of neonatal sepsis [3,27]. In this study, when patients were evaluated in terms of maternal risk factors, PROM was found to be the major risk factor with the rate of 66.7% followed by urinary tract infection 26.7 %. Similar findings were noted in studies carried out by Pokhrel B et al., from Nepal and Kayange N et al., from Tanzania [3,23]. Clinical signs and symptoms of neonatal sepsis vary [4]. In present study, the most common clinical diagnosis of neonates was neonatal pneumonia (62%). Similar to this finding, respiratory system associated disease was the most prominent clinical manifestation of sepsis in many studies [6,7,10,23]. In this study, the most performed intervention was endotracheal tube placement for mechanical ventilation with the rate of 62.8% similar to the study from Taiwan where endotracheal tube placement was the important intervention (19%) [25].

Ampicillin and gentamycin are the first-line empirical antibiotics used in NICUs. However, increasing resistance rates were reported from many countries [5,6,18,20,28]. A study from Mexico demonstrated significantly increased resistance rates of ampicillin and gentamycin for Enterobacteriaceae in LOS as 89.7% and 41.9%, respectively [11]. These findings were in line with resistance rates of ampicillin (89.5%) and gentamycin (47.4%) for Enterobacteriaceae in this study. In a study from Taiwan, lower resistance rates were detected among *E. coli* isolates for ampicillin and gentamycin as 55% and 33%, respectively [25].

Methicillin resistances in CoNS were reported from Pakistan as 75.8%, India as 61% and Egypt as 57% [2,10,13]. Also, CoNS showed high resistance to oxacillin (80%) in a study from Nepal [23]. This study revealed high resistance rates of CoNS to oxacillin as 81.2% in EOS and 83.7% in LOS, too. Obtained

results were in agreement with those found by Ozdemir AA and Elgormus Y from istanbul, Turkey [27]. In *Staphylococcus aureus* strains, methicillin resistance was detected as 38% in India and 67% in Egypt [2,10]. A lower rate (27%) was reported from Ethiopia [9]. In this study, only one *Staphylococcus aureus* strain was resistant to methicillin in LOS. All *Staphylococcal* and *Streptococcal* species were susceptible to vancomycin, teicoplanin and linezolid in this study. High susceptibility rates were found in previous reports [6,8,13,23,24]. There was only one strain of vancomycin resistant *Enterococcus* spp. in present study. This data was in contrast to data obtained by Dalal P et al., from India as they found four out of eight enterococci (50%) were resistant to vancomycin [24].

Resistance to trimethoprim/sulphamethaxazole in both gram positive and gram negative bacteria varied from 16% to 100% in studies carried out in Tanzania and India [3,21,24], it was similar with the present study where resistance rate to gram positive bacteria was less than 25% whereas it was more than 50% to gram negative bacteria.

In present study, the rate of ESBL producing isolates of Enterobacteriaceae in LOS was 63.2%. This was similar to the rate obtained in a study from India in which 67.3% of Enterobacteriaceae were ESBL producers [1]. A lower rate (40%) was determined in a study from Mexico [11]. High resistance rates of cefotaxime (63.1%) and ceftriaxone (66.9%) were also found by Najeeb S et al., from Pakistan [8]. Resistance to these antibiotics among Enterobacteriaceae varied between 44-95% in different countries [5,6,9,28].

In present study, beta-lactam/beta-lactam inhibitor antibiotics such as amoxicillin clavulanic acid had resistance rate of 78.9% among Enterobacteriaceae isolated in LOS while piperacillin-tazobactam which is in the same group had less resistance rate (26.3%). Similar results were also detected by Dalal P et al., and they used piperacillin-tazobactam as the first line empirical therapy because of high susceptibility rates [24]. Enterobacteriaceae demonstrated no resistance to carbapenems, tigecycline and colistin in this study. High susceptibility patterns of these antibiotics were also documented by recent studies [21,23,24].

Quinolones such as ciprofloxacin are not recommended for use in neonates because there is no pharmacokinetic information about dosing of ciprofloxacin in infants. These antibiotics may only be used in culture proven sepsis with bacteria not susceptible to other antibiotics [3,22]. A study from Mexico reported a resistance rate of ciprofloxacin as 44.8% for *Staphylococcus* spp in LOS [11]. Similar to this finding, present study detected the resistance rates of ciprofloxacin to CoNS in EOS and LOS as 43.8% and 42.4%, respectively. In this study, 21.1% of Enterobacteriaceae in LOS was resistant to ciprofloxacin, while only 1 (12.5%) strain of non-fermentatives showed resistance to this antibiotic. These low rates may be attributed to the fact that ciprofloxacin is not commonly administered to neonates.

Finally, CoNS and Enterobacteriaceae were common causative agents of neonatal sepsis in this study. Both gram positive and gram negative bacteria demostrated resistance to commonly used antibiotics according to data obtained in present study. On the other hand, vancomycin, teicoplanin and linezolid were the best choice of antibiotics in the treatment of methicillin resistant CoNS in early and late onset neonatal sepsis. High methicillin resistance rates may be associated with indiscriminate use of beta lactam antibiotics for prophylactic and therapeutic treatment of sepsis. Among gram negative bacteria, no resistance was observed to carbapenems.

LIMITATION

Small study population and single centered design of this study were limitations of the present study. Further multi-centered studies and large-scale participants are needed to validate findings obtained in this study.

CONCLUSION

Hospitalised neonates are at risk of EOS and LOS. Knowledge of antibiotic resistance patterns in a intensive care unit of a hospital will be helpful for rational usage of appropriate antibiotics. Also, determination of common causative agents and risk factors of neonatal sepsis is essential to manage sepsis and prevent its complications. Currently, microbiological diagnosis of sepsis with blood cultures and antibiotic susceptibility tests is gold standard but could also be improved with other rapid techniques.

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