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Original Article

Determination of the Frequency of Microbial Agents and Drug Susceptibility Pattern of the Neonatal Sepsis in the Neonatal Intensive Care Unit at Alzahra Hospital, Tabriz, Iran

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ABSTRACT

Background: Neonatal sepsis is one of the most important causes of infant mortality in developing countries. The causative organisms for sepsis are various in different regions across the world. The aim of this study was to determine the frequency of microbial agents and drug resistance pattern of the neonatal sepsis in newborns admitted to the neonatal intensive care unit (NICU) at Alzahra Hospital Tabriz, Iran.

Methods: This descriptive cross-sectional study was carried out from December 2016 to January 2018 in the NICU at Alzahra Hospital Tabriz, Iran. The medical records of all neonates admitted to the NICU were investigated using the convenience sampling method. The data were collected using a two-part demographic form. Subsequently, the data were analyzed in SPSS software (version 22.0).

Results: Out of 174 positive blood culture, 52.4% (n=92) and 46.6% (n=82) of Gram-negative (G) and Gram-positive (G) bacteria accounted for the cause of sepsis, respectively. The most common cause of early- and late-onset sepsis was Coagulase-Negative Staphylococci (CoNS), and the most common G-negative and G-positive bacteria were Acinetobacter and CoNS, respectively. The G-positive bacteria showed the most antibiotic susceptibility to Vancomycin (81.45%), Ampicillin (52.15%), and Imipenem (47.32%). On the other hand, the highest drug susceptibility in G-negative bacteria was related to antibiotics, such as Amikacin (73.64%), Imipenem (56.36%), and Ciprofloxacin (52.44%). Moreover, the most antibiotic resistance was associated with Oxacillin (100%), Tetracycline (100%), and Ciprofloxacin (44.4%).

Conclusion: The CoNS is the main cause of early- and late-onset sepsis among the neonates admitted to the NICU at Alzahra Hospital, Tabriz, Iran. G-positive and G-negative as causative agents of sepsis showed the highest susceptibility to Vancomycin and Amikacin, respectively.

Keywords: Antibiotic treatment, Drug resistance, Microbial agents, Neonatal sepsis

Introduction

Neonatal sepsis is defined as a type of systemic inflammatory response syndrome that occurs in the presence or secondary to a proven or suspected infection which is characterized by general signs of infection and tissue damage in the first 28 days of birth (1, 2). The incidence of sepsis in the neonate is greater than that at any other period of life. The prevalence of neonatal sepsis in developed countries is equivalent to 1-4 per 1,000 live births, whereas it is reported to be nearly 10 times higher in developing countries (3, 4). Some other population-based studies from developing countries have reported clinical sepsis rates ranging from 49-170 per 1,000 live births (5, 6).

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Neonatal sepsis is still one of the leading causes of mortality and disability worldwide with the mortality rate of around 30-50% in developing countries (7-9).

Neonatal sepsis is classified into two groups, namely early-onset neonatal sepsis (EONS) which occurs at 72 hours after birth and late-onset neonatal sepsis (LONS) which takes place within 72-30 hours after birth (10-13). Different bacterial pathogens are involved in neonatal sepsis. Although Gram-negative (G) bacteria was reported as the main cause of neonatal sepsis in previous studies, especially in developed countries, Coagulase Negative Staphylococcus (CoNS) has been considered as the main cause of neonatal sepsis in many recent studies (14,15).

Although diagnostic methods differ in the neonatal sepsis, the treatment of sepsis in neonates includes supportive care and antibiotic therapy followed by early empirical treatment and specific treatment of microorganisms (16). Antibiotics are among the most widely used drugs in the Neonatal Intensive Care Unit (NICU) (17-19), and their prolonged use is associated with increased mortality rate and necrotizing enterocolitis in newborns (20, 21). On the other hand, antibiotic therapy can change the microbial flora of the infant and potentially predispose them to opportunistic infections, which leads to the development of antibiotic-resistant organisms (22).

The pattern of the bacterial agent is responsible for the neonatal septicemia over time, and the use of different antibiotics results in resistance to them. Additionally, the prevalence of septicemia is different from one community to another; therefore, consistent epidemiological monitoring in the hospital, especially at NICUs, is essential to have an antibiotic guideline based on the relevant microbial agents.

Given the importance of early diagnosis and treatment of neonatal sepsis and the development of a clinical guideline for the antibiotics therapy, there is a need to identify the patterns of causative organisms for sepsis and antibiotic susceptibility patterns in each hospital. Accordingly, this study aimed to determine the frequency of microbial agents and drug susceptibility pattern of the neonatal sepsis among newborns admitted to the NICU at Alzahra Hospital Tabriz, Iran.

Methods

Study design and population

This descriptive cross-sectional study was conducted from December 2016 to January 2018 in the NICU at Alzahra teaching Hospital, Tabriz, Iran. This study included all medical records of the neonates admitted to the NICU at Alzahra teaching Hospital, Tabriz, Iran.

Methods

During a year, all sepsis agents, including blood, urine, and cerebrospinal fluid (CSF) cultures, were obtained by catheterization or suprapubic in the NICU at Alzahra Hospital, Tabriz, Iran, and recorded in the medical records of the neonates to obtain the frequency of microbial agents and their microbial resistance patterns. The neonatal period of the full-term and pre-term infants is defined as the first 28 days of life and gestational age of 44 weeks. Gestational ages were confirmed by the mother using the last date of the menstrual period and obstetrical ultrasonography.

Data collection instruments

Data were collected using a researcher-made demographic form which consisted of two parts. The first part sought information as gender, weight, and gestational age, and the second part asked for data regarding neonatal sepsis agents, including blood, urine, and CSF cultures.

Ethical considerations

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (IR.TBZMED.REC.1394.1045). Moreover, the required official permission was obtained from the hospital where the study was conducted. After inviting the families by telephone, informed written consent was obtained from the parents, and they were all informed of the confidentiality of the data as well as the research procedures and objectives.

Inclusion and exclusion criteria

All causative organisms for sepsis, including positive blood cultures and drug susceptibility pattern were extracted from the neonates' medical records. Blood culture was taken according to the standard method recommended by the World Health Organization (23). The blood samples were taken from a peripheral vein or an artery. In order to prevent the possibility of contamination with real positive blood culture, the blood sampling was carried out using a sterile technique. After sterilization, the BACTEC technique (BACTEC 9120 device manufactured by BD Inc) was used to obtain bacterial growth and drug sensitivity after 24-48-hour incubation. Moreover, positive blood cultures were collected

after 48 hours. To ensure the accuracy of positive blood culture, especially in CoNS, the result was matched to the clinical symptoms of the patient, and if the answer was wrong, the blood culture was sent again.

The CSF was collected using a sterilized method and urine samples were taken by catheterization or suprapubic. This study included all infants born at this center or those who were referred to this center with positive blood culture and clinical symptoms in favor of both EONS and LONS. On the other hand, neonates with congenital anomalies and/or dysmorphic disorder were excluded from the study.

Statistical analyses

The data were analyzed using SPSS software (version 22.0) through descriptive statistics, including mean, standard deviation, frequency, and percentage.

Results

In this study, 174 neonates had a positive blood culture, and 107 (61.5%) cases were male. The mean ages of the neonates were 12.93 and 8.50 days for males and females, respectively (age range: 1 to 74 day). Table 1 summarizes other demographic characteristics of the neonates. The results of this study showed that in neonates with positive blood culture, G-negative bacteria, including Acinetobacter (19.8%) and Pseudomonas (11%) as well as Gram-positive (G) bacteria, such as CoNS (35.2%) and Streptococcus pneumonia (0.6%) were the highest and lowest bacterial microorganisms causing neonatal sepsis, respectively (Table 2).

Furthermore, these results indicate that G-positive bacterial agents, especially CoNS, were the causative agent of neonatal sepsis in infants admitted to the NICU at Alzahra Hospital in Tabriz. Based on the results of this study, out of 147 newborns with positive blood cultures, 57 and 101 neonates had EONS and LONS, respectively. Therefore, the most common cause of neonatal sepsis in this center was EONS and related microbial agents. The frequency of other bacterial agents in EONS and LONS has been shown in Table 3.

Table 1. Demographic characteristics of newborns

Demographic Characteristics	Frequency		Percent (%)
Gender			
Male	107		61.5
Female	67		38.5
Type of Delivery			
Natural vaginal delivery	98		56.3
Caesarian section	76		43.7
Age of sepsis			
Before 72 hours	24		13.8
After 73 hours	150		86.2
	Minimum	Maximum	Mean ± S.D
Gestational age	24	39	32.13±2.94
Birth weight	530	4750	1834.55±738.04

Table 2. Frequency of various bacteria causing neonatal sepsis in neonates admitted to the neonatal intensive care unit at Alzahra Hospital, Tabriz,Iran

Type of bacteria		N	Percentage (%)
Gram-negative	Acinetobacter	33	19.8
	Klebsiella	22	12.5
	Enterobacter	20	11.4
	E-coli	9	5.2
	Citrobacter	6	3.4
	Pseudomonas	2	1.1
Total		92	53.4
Gram-positive	Coagulase-negative staphylococci (CoNS)	62	35.2
	Enterococcus	10	5.7
	Staphylococcus aureus	5	2.8
	Group B Streptococcus (GBS)	3	1.7
	Group A streptococcus (GABHS)	1	0.6
	Streptococcus Pneumonia	1	0.6
Total		82	46.6

Additionally, figures 1 and 2 illustrate that CoNS is the most common bacterial cause of both EONS (38.6%) and LONS (31.6%) in this study. The prevalence of other bacterial agents in EONS and LONS is shown in figures 1 and 2. According to the results obtained from this study, Vancomycin (81.45%) and Ceftriaxone (3.3%) obtained the highest and lowest level of susceptibility to G-positive bacterial agents, respectively. Moreover, regarding the sensitivity

Table 3. Frequency of bacterial agents in early-onset sepsis and late-onset sepsis

Bacterial Agents		EONS		LONS	
		N	%	N	%
Gram negative	Klebsiella	6	10.5	15	14.9
	Enterobacter	6	10.5	14	13.9
	Acinetobacter	5	8.8	23	22.8
	E-coli	4	7	5	5
	Citrobacter	0	0	5	5
	Pseudomonas	2	3.5	0	0
Gram positive	Coagulase-negative staphylococci (CoNS)	22	38.6	32	31.6
	Enterococcus	6	10.5	3	3
	Staph Aureus	2	3.5	3	3
	Group B streptococcus (GBS)	2	3.5	1	1
	Group A streptococcus(GABHS)	1	1.8	0	0
	Streptococcus Pneumonia	1	1.8	0	0
Total		57	100	101	100

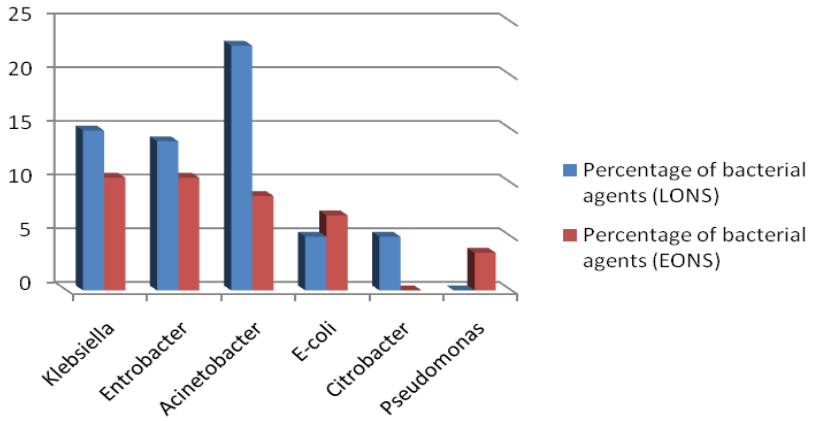

Figure 1. Comparison of frequency of Gram-negative microbial agents of neonatal sepsis in newborns admitted to Alzahra Hospital, Tabriz, Iran during 2016-2018

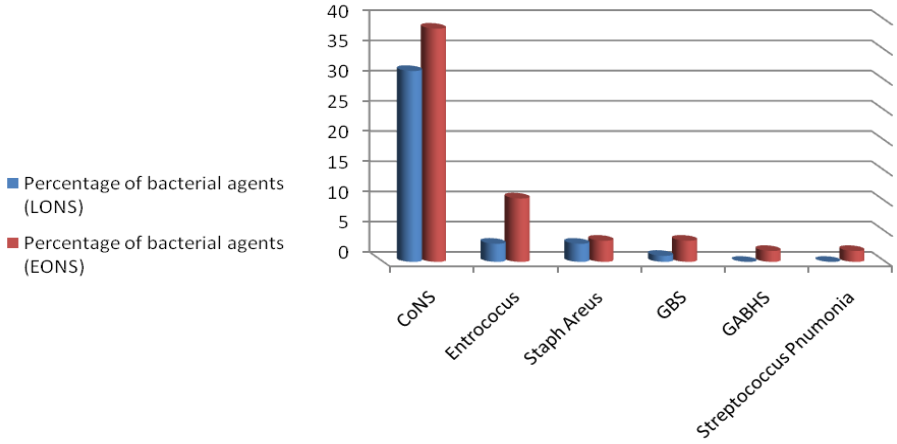

Figure 2. Comparison of frequency of Gram-positive microbial agents of neonatal sepsis in newborns admitted to Alzahra Hospital, Tabriz, Iran during 2016-2018
CoNS: Coagulase-negative staphylococci
GBS: Group B streptococcus
GABHS: Group A streptococcus

Table 4. Frequency distribution of drug sensitivity of Gram-positive and Gram-negative bacteria in sepsis among neonates admitted to the neonatal intensive care unit at Alzahra Hospital, Tabriz, Iran during 2016-2018

Germs		Antibiotics								
		Ampicillin %	Gentamicin %	Amikacin %	Vancomycin %	Imipenem %	Ciprofloxacin %	Cotrimoxazole %	Tetracycline %	Ceftizoxim %
Gram positive	*CoNS	41.9	30.6	3.2	75.8	22.6	19.4	3.2	6.5	14.5
	Enterococci	80	30	0	90	60	50	10	10	50
	**GBS	66.7	33.3	0	100	66.7	66.7	33.3	33.3	33.3
	Staph aureus	20	0	0	60	40	40	20	20	20
Mean sensitivity		52.15	23.47	0.8	81.45	47.32	44.02	16.62	17.45	29.45
Gram negative	Acinetobacter	3	18.2	91.9	6.1	6.1	3	0	12.1	18.2
	Klebsiella	13.6	27.3	81.8	0	77.3	63.6	22.7	13.6	50
	Enterobacter	20	70	50	0	65	80	40	5	40
	Escherichia. coli	0	33.3	77.8	0	66.7	33.3	0	11.1	66.7
	Citrobacter	0	16.7	66.7	0	66.7	83.3	33.3	50	50
Mean sensitivity		7.32	33.1	73.64	1.22	56.36	52.64	19.2	18.36	44.98

* CoNS: Coagulase-negative staphylococci
** GBS: Group B streptococcus

to G-negative bacterial agents, Amikacin (73.64%) and Ampicillin (7.32%) obtained the highest and lowest level in this regard, respectively (Table 4).

Discussion

Although progress in the care of the neonatal specialty has led to a reduction in ENOS in term neonates, the preterm newborns are still at high risk for ENOS and its complications. Very Low Birth Weight (VLBW) infants are also at risk for LONS. Sepsis surviving infants may also be affected by severe central nervous system infections due to septic shock, persistent pulmonary hypertension of the newborn, and severe pulmonary parenchyma associated with hypoxia (24). The incidence of sepsis in the neonate is greater than that at any other period of life. The aim of this study was to determine the frequency of microbial agents and drug resistance pattern of the neonatal sepsis among neonates admitted to the NICU at Alzahra Hospital, Tabriz, Iran.

According to the results of this study, among G-positive and G-negative bacteria, CoNS and Acinetobacter were the causative agents of neonatal sepsis, respectively. Few pieces of research were conducted on the etiologic cause of neonatal sepsis in Iran. In a study conducted by Ghotaslou et al. in the NICU at Tabriz Children's Hospital (a referral center for outborn neonates) from 2002 to 2004, CoNS (68.6%) and Klebsiella pneumonia (31.4%) were determined as the most common causes of neonatal sepsis, respectively (25).

Moreover, Khalilimatinzadeh et al. revealed that CoNS (44.7%) and Escherichia coli (23.7%) were the most common microorganisms in two NICUs at Tehran, Iran, from 2001 to 2005 (26). In the same line, according to the results of a two-year study conducted by Dezfoulimanesh et al. in the NICU at Imam Reza Hospital in Kermanshah, staphylococcus aureus and Klebsiella pneumonia were the most common causes of septicemia (27). Although G-negative bacteria has been reported as the most common cause of neonatal sepsis in previous studies, as well as in developed countries, the role of CoNS has been confirmed as the main cause of neonatal sepsis in many recent studies (28). The results of these studies are consistent with the findings obtained from the present study.

Unlike the reports in western countries, the incidence of sepsis caused by group B streptococcus (GBS) was very low (1.7%) in the present study (29, 30) which was consistent with findings of other studies conducted in Iran. Therefore, it seems that GBS is not a major colonization bacteria in the genitalia of the pregnant mother or there may be a diagnostic problem in the finding of microorganism (14). Additionally, Rafati et al. performed a study in 2013 entitled "Determination of frequency and antibiotic resistance of common bacteria in late-onset sepsis at the neonatal ward in Booali-Sina Hospital, Sari, Iran". According to the result, Staphylococcus aureus, Klebsiella pneumonia, and E.coli were regarded as the most common microorganism agents (9). The results of these studies contradict the findings obtained from this study. The difference in the results suggests that the microbial agents of newborn sepsis may vary in different geographical locations and may change over time. Therefore, various studies in different places and times are necessary in this regard.

In the present study, CoNS was the most prevalent neonatal sepsis microorganism both in EONS and LONS. Similarly, Gheibi et al. conducted a 50-month study (From 2003 to 2007) in the NICU at Imam Khomeini Educational Hospital in Urmia. The results showed that CoNS was the most common cause of both EONS (48.8%) and

LONS (69.8%) (14). Similar findings were obtained in other countries (i.e., Egypt, China, Mexico, and Kenya) in this regard (10, 31-33). These findings were expected for LONS since Alzahra Hospital is a referral center for high-risk pregnancies in the northwest of Iran, and most of the premature and VLBW infants were admitted there. However, it was unexpected to find the CoNS as a causative organism of EONS. Therefore, the neonates' medical records and other laboratory data, as well as the colony counts, were reviewed to find probable blood sampling errors.

The laboratory findings, including complete blood count, C-reactive protein, and colony count were matched with blood cultures to minimize the error of the contamination reporting. Subsequently, 4 and 1 EONS and LONS were excluded, respectively, to increase the validity of the report. Another result obtained from this study showed that Enterococcus (10.5%) and Klebsiella (10.5%) were the most prevalent G-negative causative bacteria for EONS after CoNS, which itself requires the investigation of the bacterial colonization in pregnant mothers. However, Acinetobacter and Klebsiella were the second and third most prevalent G-negative bacteria in the LONS. Among G-positive strain, the highest susceptibility rate was found in Vancomycin, Ampicillin, Imipenem, and Ciprofloxacin. Moreover, the highest susceptibility rate in G-negative strains was also observed in Amikacin, Imipenem, and Ciprofloxacin.

Similarly, Gheibi et al. in their study showed that G-positive bacteria had the highest susceptibility to Vancomycin (90%), Ciprofloxacin (92.8%), and Imipenem (67.7%) and G-negative bacteria were highly susceptible to Ciprofloxacin, Amikacin, and Imipenem (14). However, in the study carried out by Dezfulimanesh et al., G-positive bacteria showed the highest antibiotic- susceptibility in Ciprofloxacin, Imipenem, Ceftriaxone, and Cotrimoxazole. Furthermore, the highest drug susceptibility in G-negative bacteria was related to antibiotics, such as Ciprofloxacin, Imipenem, Cotrimoxazole, Cefixime, and Ampicillin (27).

The results of this study showed that most of the CoNS still were susceptible to Vancomycin, Ampicillin, and Gentamicin; however, it was found that 24% of CoNS was not sensitive to Vancomycin. Unfortunately, it was impossible to measure the minimal inhibitory concentration level in this study. Moreover, Foruzandeh et al. (2017) conducted a study in the NICU at a hospital in Kerman, Iran. The results showed that CoNS in that setting had the highest rate of susceptibility to Vancomycin (100%), Novobiocin (100%) and Gentamicin (83.3%) (28). This finding emphasizes minimizing the time of prescribing Vancomycin to prevent more resistance in the unit.

Methicillin-resistance was not evaluated in this study, which itself should be considered seriously. However, the Ampicillin-resistance rate of G-positive bacteria (i.e., about 47.85%) is a cause of concern. Given the previous protocol in the prescribed dose range for Ampicillin (i.e., 50 mg/kg per group of infants based on the day after birth and birth weight), a revision of the recommended dose was performed in this study (i.e., 100 mg/kg) (34).

Limitations of the study

The limitations of this study included the incomplete data in medical records of some neonates and lack of access to complete information. On the other hand, since the microbial causes of the neonatal sepsis vary in different places and times, similar studies are recommended to be conducted in different regions at different times.

Conclusion

The results of this study showed that CoNS was the main cause of ENOS and LONS among the neonates admitted to the NICU at Alzahra Hospital, Tabriz, Iran. In addition, the highest rate of susceptibility to Vancomycin and Amikacin was observed in G-positive and G-negative causative bacteria for sepsis, respectively. Therefore, given the causative microbial agent of neonatal sepsis and antibiotic susceptibility, suitable drugs could be selected to help treat this neonatal infection more appropriately.

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Conflicts of interests

The authors declare no conflict of interest regarding the publication of the study.

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