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Prevalence and Antibiotic Resistance of Neonatal Sepsis Pathogens in Neyshabour, Iran

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Abstract

Background: Neonatal sepsis is a systemic inflammatory response syndrome that is secondary to infection. It is a major cause of neonatal mortality in the world, particularly in developing countries. A definitive diagnosis requires the isolation of pathogens from a normally sterile body site, including blood, cerebrospinal fluid and urine. Empirical antibiotic therapy is based on the physician's knowledge of the anticipated bacterial species and their expected antibiotic susceptibilities.
Objectives: The aim of this study was to determine the prevalence and evaluate the antimicrobial susceptibility patterns of bacterial infections at a neonatal unit.
Patients and Methods: This study was conducted at the neonatal intensive care unit and neonatal ward of Hakim hospital, Neyshabour, Iran. Blood, cerebrospinal fluid (CSF) and urine specimens were collected before institution of empirical antibiotic therapy. Antibiotic resistance pattern of the isolates was studied by the disc diffusion technique.
Results: Coagulase-negative staphylococci (CoNS) were the most prevalent pathogens isolated from blood specimens in early and late-onset disease. *Escherichia coli* and *Klebsiella* were the most causative pathogens in early and late-onset urinary tract infections. They had high resistance to our empirical antibiotic regimens. Prevalence of bacterial meningitis was low in our study.
Conclusions: Due to the increasing resistance of pathogens to usual empirical antibiotics, it is reasonable to stress upon preventive measures, so that a minimum number of neonates develop sepsis.

Keywords: Newborn, Sepsis, Drug Resistance, Microbial Sensitivity Tests

1. Background

Neonatal sepsis is a systemic inflammatory response syndrome that is secondary to infection (1). It is a major cause of neonatal mortality in the world, particularly in developing countries and is responsible for 30% - 50% of infant mortality in these communities (2).

Neonatal sepsis is categorized according to the infant's postnatal age at onset of disease. Most clinicians define early-onset sepsis (EOS) as that occurring during the first 72 hours of life and late-onset sepsis (LOS) occurring after 72 hours (1).

A definitive diagnosis requires the isolation of pathogen from a normally sterile body site, including blood, cerebrospinal fluid and urine (1).

Owing to non-specific symptoms, prematurity of infant's immune system and high neonatal mortality rate due to infections, before preparing the culture and definitive diagnosis, experimental antibiotics are prescribed for these infants (3).

Empirical antibiotic therapy is based on the physician's knowledge of the anticipated bacterial species and their expected antibiotic susceptibilities (4).

Due to the consumption of antibiotics over time and in different countries (developed or developing), changes in prevalence and sensitivity of these bacteria have occurred; for example, resistant bacterial strains include gentamicin-resistant *Klebsiella* species, third-generation cephalosporin-resistant gram-negative organisms, and methicillin-resistant staphylococci (5, 6).

Therefore, continuous epidemiologic monitoring by repeated local revisions of susceptibility patterns to antibiotic agents is necessary to establish a rational treatment strategy (7).

2. Objectives

According to the importance of the early diagnosis and selection of appropriate empirical antibiotics for neonatal sepsis, to bring down the morbidity and mortality substantially, we aimed to determine the frequency and antibiotic resistance of pathogens at the neonatal intensive-care unit (NICU) and neonatal ward of our hospital.

3. Patients and Methods

Hakim hospital is a teaching hospital affiliated to Neyshabour University of Medical Sciences, which provides high-risk obstetric services, with 9000 to 9500 annual deliveries.

In this retrospective cross-sectional study, pertinent data were obtained from the records of all newborn infants cared for at the neonatal intensive care unit and neonatal ward of Hakim hospital between 23 September 2013 and 22 September 2014.

At the hospital, before institution of antibiotic therapy, a single specimen of half to one milliliter of blood was collected aseptically (from peripheral vessels or from central catheters at the time of first insertion) for culture from all infants, who had risk factors for infection and relevant clinical symptoms. The specimens were inoculated with respect to aseptic principles into Triptycase soy broth culture medium. The inoculated bottles were transported immediately to the department of microbiology and incubated at 37°C for 48 hours. Next, subcultures were done on chocolate agar plates (for gram positive bacteria) and MacConkey plates (for gram negative bacteria). The isolated colonies were identified by their colonial morphology, gram stain, conventional biochemical tests (based on the methods of Cowan et al.) and by using API 20 E galleries (8).

Cerebrospinal fluid (CSF) was obtained when clinically indicated. The CSF specimen was plated directly on blood agar plate (BAP), chocolate agar plate (CAP) and eosin methylene blue media (EMB) and used for gram staining.

In cases of suspected urinary tract infection (UTI), urine specimen for culture was obtained either with the aseptic method or with a urine bag and plated onto a blood agar plate (BAP), an EMB media, and used for the gram stain.

Antibiotic resistance pattern of the isolates was studied using the modified kirby baur disc diffusion technique (9).

The results were interpreted according to the clinical and laboratory standards institute (CLSI) (10) and when susceptibility was intermediate, the bacteria were considered resistant.

After obtaining culture specimens, we began the prescription of antibiotics, ampicillin (Ampivil, 250 mg) and amikacin (Ipacin, 100 mg) for early onset and ampicillin plus cefotaxime (Cephotax, 500 mg) for late onset sepsis with a dose based on age and weight of neonate (11).

The SPSS version18 statistical software was used to perform the statistical analysis of this study.

4. Results

4.1. Blood

Out of 1111 blood samples, one hundred and thirty two samples (11.9%) yielded the growth of some bacterial pathogens, from which 86 cases (65%) were due to early onset and 46 cases (35%) due to late onset sepsis.

Among the studied neonates for early onset of sepsis, 66.3% were male and 33.7% were female (male to female ratio of 57:29), while in late onset sepsis male to female ratio was 22:24.

The most common organisms causing early-onset disease were Coagulase-Negative Staphylococci (CoNS) (33.7%), *Streptococcus hemolyticus* (19.8%) and *Staphylococcus aur*eus (17.4%). *Escherichia coli* and *Klebsiella* were the most prevalent gram-negative pathogens in early onset sepsis (Table 1).

Coagulase-Negative Staphylococci were also the most prevalent in late-onset disease (34.8%), followed by *staphylococcus aureus* (21.7%) and gram-negative organisms (19.6%). *Pseudomonas aeruginosa* was the most prevalent gram-negative pathogen in late onset sepsis (three out of nine cases) (Table 2).

The CoNS were significantly resistant to antibiotics of our empirical treatment. In total, 40% were resistant to ampicillin, 20% to amikacin and 31% to cefotaxime, especially in the case of late onset sepsis for the first two (Tables 1 and 2). Twenty-six percent of cases (24% in EOS and 31% in LOS) were resistant to vancomycin.

Twenty-four percent of *Staphylococcus aureus* were resistant to ampicillin but none of them were vancomycin resistant.

In the case of *Streptococcus hemolyticus*, 47.8% were resistant to ampicillin and 8.7% to vancomycin.

Furthermore, 45.5% of gram-negative bacteria were resistant to amikacin, 36.4% to gentamicin and 13.6% to cefotaxime.

4.2. Urine

We had 414 neonates with clinical findings suggesting urinary tract infection (UTI) during the study period, among them, 85 cases (20.5%) had a positive urine culture, 22 (26%) infants were less than three days old and 63 (74%) infants were above the age of three days.

Male to female ratio in early onset sepsis due to UTI was 15:7 and for late onset cases was 39: 24.

The most common organisms causing early-onset disease were *E. coli* (36.3%), *Klebsiella* (31.8%), *Enterobacter* and *Enterococci* (each 13.6%).

Escherichia coli and *Klebsiella* were also the most causative pathogens in late-onset disease (34.9% and 31.7%,

Table 1. Antibiotic Resistance of Common Organisms in Blood Culture (Early Onset Septicemia 86)[a]

Antibiotic	CoNS29 (33.7)	*Staphylococcus aureus* 15 (17.4)	*Streptococcus hemolyticus* 17 (19.8)	Strep Non hemolyticus 3 (3.5)	Gram negative bacilli 13 (15.1)
Ampicillin	10 (34.5)	5 (33.3)	8 (47)	1 (33.3)	6 (46.1)
Amikacin	6 (20.7)	1 (6.6)	4 (23.5)	1 (33.3)	6 (46.1)
Gentamicin	11 (37.9)	2 (13.3)	8 (47)	2 (66.6)	5 (38.5)
Cefotaxime	10 (34.5)	1 (6.6)	3 (17.6)	0	1 (7.7)
Vancomycin	7 (24.1)	0	2 (11.8)	0	NT
Imipenem	9 (31)	2 (13.3)	4 (23.5)	0	NT
Ciprofloxacin	4 (13.8)	1 (6.6)	1 (5.6)	0	1 (7.7)
Penicillin	12 (41.4)	7 (46.6)	7 (41.2)	0	NT

Abbreviations: CoNS, Coagulase-Negative Staphylococci; NT, Not Tested.
[a]Values are expressed as No. (%).

Table 2. Antibiotic Resistance of Common Organisms in Blood Culture (Late Onset Septicemia 46)[a]

Antibiotic	CoNS 16 (34.8)	*Staphylococcus aureus* 10 (21.7)	*Streptococcus hemolyticus* 6 (13)	Strep Non Hemolyticus 3 (6.5)	Gram Negative 9 (19.6)
Ampicillin	8 (50)	1 (10)	3 (50)	1 (33.3)	5 (55.5)
Amikacin	3 (18.8)	2 (20)	0	3 (100)	4 (44.4)
Gentamicin	3 (18.8)	5 (50)	0	2 (66.6)	3 (33.3)
Cefotaxime	4 (25)	3 (30)	1 (16.6)	0	2 (22.2)
Vancomycin	5 (31.3)	0	0	0	NT
Imipenem	6 (37.5)	4 (40)	1 (16.6)	2 (66.6)	NT
Ciprofloxacin	4 (25)	1 (10)	1 (16.6)	0	0
Penicillin	6 (37.5)	7 (70)	3 (100)	0	3 (33.3)

Abbreviations: CoNS, Coagulase-Negative Staphylococci; NT, Not Tested.
[a]Values are expressed as No. (%).

respectively) followed by *Enterobacter* (9.5%) and *Staphylococcus aureus* (7.9%).

Of the *E. coli* isolates, 36.6% were resistant to ampicillin and amikacin and 26.6% to cefotaxime, as empirical antibiotics, which were used at our hospital. *Klebsiella* isolates, as the second leading cause of early and late onset disease, were 22.2%, 25.9% and 18.5% resistant to ampicillin, amikacin and cefotaxime, respectively (Tables 3 and 4).

4.3. Cerebrospinal Fluid

During the study period, 101 CSF samples were obtained, out of which three samples (2.9%) were positive.

Two infants were less than three days old and the third infant was above the age of three days. Two were male and one was female.

In the first case, *Klebsiella* was isolated from CSF and was resistant to ceftriaxone, ceftizoxime, amikacin and gentamicin. *Escherichia coli* was yielded from blood culture at the same time.

In the other case of early onset disease, *Staphylococcus epidermidis* was isolated from CSF, which was sensitive to all antibiotics that were tested (including amikacin, gentamicin, cefotaxime and vancomycin). *Enterobacter* grew in blood culture, simultaneously.

In the third case, a 21-day-old boy, Staphylococcus haemolyticus, which was resistant only to penicillin, was yielded from CSF culture and the concomitant blood culture was negative.

5. Discussion

5.1. Blood

The prevalence of culture positive (proven) neonatal sepsis is different in various studies. While in some studies it has been reported as high as 44.7% (Ethiopia) (12) and

Table 3. Antibiotic Resistance of Common Organisms in Urine Culture (Early Onset Urinary Tract Infection 22)[a]

Antibiotic	*E. coli* 8 (36.4)	*Klebsiella* 7 (31.8)	*Enterobacter* 3 (13.6)	*Enterococci* 3 (13.6)	Gram positive bacilli 1 (4.5)
Ampicillin	4 (50)	2 (28.6)	1 (33.3)	1 (33.3)	1 (100)
Amikacin	5 (62.5)	3 (42.8)	2 (66.6)	1 (33.3)	-
Gentamicin	2 (25)	2 (28.6)	2 (66.6)	1 (33.3)	-
Cefotaxime	3 (37.5)	2 (28.6)	1 (33.3)	2 (66.6)	1 (100)
Ceftriaxone	4 (50)	5 (71.4)	2 (66.6)	2 (66.6)	1 (100)
Cefixime	2 (25)	3 (42.8)	2 (66.6)	2 (66.6)	1 (100)
Vancomycin	NT	NT	NT	2 (66.6)	1 (100)
Imipenem	-	-	-	1 (33.3)	-
Nitrofurantoin	3 (37.5)	2 (28.6)	2 (66.6)	NT	NT
Nalidixic acid	1 (12.5)	-	-	NT	NT

Abbreviations: CoNS, Coagulase-Negative Staphylococci; NT, Not Tested.
[a]Values are expressed as No. (%).

Table 4. Antibiotic Resistance of Common Organisms in Urine Culture (Late Onset Urinary Tract Infection 63)[a]

Antibiotic	*E. coli* 22 (34.9)	CoNS 3 (1.9)	*Klebsiella* 20 (31.7)	*Enterobacter* 6 (9.5)	*Staphylococcus aureus* 5 (1.3)
Ampicillin	7 (31.8)	2 (66.6)	4 (20)	1 (16.6)	1 (20)
Amikacin	6 (27.3)	1 (33.3)	4 (20)	1 (16.6)	-
Gentamicin	3 (13.6)	-	4 (20)	1 (16.6)	1 (20)
Cefotaxime	5 (22.7)	-	3 (15)	1 (16.6)	1 (20)
Ceftriaxone	8 (36.4)	3 (100)	11 (55)	1 (16.6)	3 (60)
Cefixime	12 (54.5)	-	5 (25)	2 (33.3)	3 (60)
Vancomycin	NT	-	NT	NT	1 (20)
Imipenem	1 (4.5)	2 (66.6)	-	-	-
Nitrofurantoin	5 (22.7)	NT	7 (35)	1 (16.6)	NT
Nalidixic acid	4 (18.2)	NT	-	-	NT

Abbreviations: CoNS, Coagulase-Negative Staphylococci; NT, Not Tested.
[a]Values are expressed as No. (%).

45.9% (Nigeria) (13), in other studies it is about 20% (14). It was 11.9% in this study, which can be explained by the different clinical criteria for suspicion of sepsis and taking blood samples, the technique of culture (traditional culture vs. BACTEC), quality of life and measures of health care and hospital services in various countries (15, 16).

In our study, early onset sepsis was more prevalent than late onset sepsis (65% vs. 35%), which is in agreement with previous reports (17). However, the opposite was documented in some other previous studies (18).

The high male to female ratio in early onset sepsis corresponded with previous studies, which have considered the male gender as a risk factor for neonatal septicemia (19).

In our study, gram-positive cocci, specifically CoNS, were more common in both EOS and LOS compared to fram-negative bacteria. Similar findings were obtained in other studies from different countries such as Egypt, China, Mexico, south Africa and Kenya (18). High rates of CoNS infections were reported in the middle east, south-east Asia and Latin America (20).

In previous studies from other regions of our country, different causative pathogens were reported as the most prevalent causes of neonatal bacteremia. In some studies, gram-negative bacteria such as *Klebsiella* were the predominant cause of neonatal sepsis, especially in LOS, while in other studies CoNS were more common to cause neonatal sepsis (14, 21). In one study, the most common isolated or-

ganism from blood cultures was Flavobacterium (22).

The extensive use of invasive devices to care for immunologically-immature neonates, especially preterm and low birth weight (LBW), is the main cause of CoNS bacteremia in NICU; determination of the identity of CoNS isolates whether being true pathogens or contaminants is still problematic (16). It cannot be ruled out that some CoNS isolates might have been a consequence of a contamination. However, the results of our study showed the pattern of bacterial susceptibility to antibiotics and therefore such cases, if present, are pertinent for the study (7).

Ampicillin and aminoglycoside (amikacin) are the first-line empirical antibiotics used in our NICU for early onset sepsis. Coagulase-Negative Staphylococci, as the most prevalent organism, are 40% resistant to ampicillin and 20% resistant to amikacin. The following two other prevalent causes (*Streptococcus haemolyticus* and *Staphylococcus aureus*) are also relatively resistant to this regimen (Table 1). Despite other previous studies (7, 14, 17, 23, 24), 26.6% of CoNS and 8.7% of *Streptococcus hemolyticus* strains were also partially or completely resistant to vancomycin. Overall, 13.6% and 50% of gram negative bacteria, as the third leading cause of late onset sepsis, were respectively resistant to cefotaxime and ampicillin, the two antibiotics in our empirical treatment for late onset sepsis.

Despite the high resistance of common pathogens to empirical antibiotics used at our hospital, second line antibiotics such as vancomycin, imipenem and quinolones could not be recommended to be used as empirical antibiotics for reserving certain drugs to combat against emerging resistant strains (7). Thus, we should stress more upon preventive measures, so that a minimum number of our neonates develop sepsis. These preventive measures should focus on recognition of high-risk infants, strict asepsis during labor and early institution of exclusive breast-feeding (25).

5.2. Urine

In our study we found that the prevalence of proven UTIs among clinically suspected cases of UTI was 20.5%, which corresponds with the results of some previous studies (26). However, the results in this case are also very different from 7.5% (27) to 41.3% (28).

The difference in the prevalence of UTI in various studies may be explained by the selection of neonates from different locations. While in some studies patients were selected only from NICU admitted neonates, who are more susceptible to infection, in other studies nursery admitted neonates (as our study) and even outpatient neonates were also included. The difference probably reflects variations in population characteristics and in predisposing factors (28). Method of sample collection (only suprapubic aspiration or samples collected with a bag) was also very important.

Because several urine samples in our study were collected with a urine bag, it is possible that some positive culture results are due to contamination but as mentioned for CoNS isolates in blood culture, our results demonstrate the pattern of bacterial susceptibility to antibiotics and therefore isolates, which were isolated as a consequence of a contamination, are still pertinent for the study.

In our study, there was a higher prevalence of UTI in males compared with females and this is in agreement with similar studies, which found that males are more affected than females with UTI in the neonatal period (29).

In our study *E. coli* and *Klebsiella* were the most common causes of UTIs in early and late onset sepsis, which is consistent with other previous studies (27, 28, 30), yet unlike previous studies, they have high resistance rates to aminoglycosides (Tables 3 and 4). In the case of cephalosporins, they have acceptable susceptibility to cefotaxime (73.4% for *E. coli* and 81.5% for *Klebsiella*), but are resistant to ceftriaxone in 40% and 59.2% of cases, respectively which is also in contrast with previous studies (30-33). Choosing cefotaxime as an appropriate empirical antibiotic for the treatment of UTI, before obtaining the microbiologic results, seems reasonable in our hospital.

5.3. Cerebrospinal Fluid

In this study, the prevalence of bacterial meningitis in suspected cases was 2.9%. Two previous studies have reported prevalences of 4.4% and 5.4% for culture-proven bacterial meningitis in suspected neonates (34, 35).

It is likely that the incidence of bacterial neonatal meningitis remains underestimated. Several studies have highlighted this underestimation, both for early- and late-onset meningitis (36). Nearly 30% of bacterial meningitis cases in infants are not diagnosed when only one blood culture is performed to confirm neonatal infection (37). The low prevalence can also be due to antibiotic administration before CSF culture and the lack of investigation for anaerobic bacteria.

Previous studies have shown that bacterial meningitis is slightly more common in boys, which is consistent with our results (38).

Klebsiella and strains of *Staphylococcus* are common pathogens for neonatal meningitis (39).

In other studies it has been determined that 62% of patients had a concomitant-positive blood culture, and in 3.5% of cases, as in our study, the organisms isolated were discordant; in each case, the CSF pathogen required different antimicrobial therapy than the blood pathogen (40).

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Footnotes

Authors' Contribution: Homayoon Behmadi: acquisition of data, analysis, and interpretation of data, and drafting of the manuscript; Abasalt Borji: critical revision of the manuscript for important intellectual content, administrative, technical and material support, and study supervision; Azadeh Taghavi-Rad: acquisition of data, analysis and interpretation of data, and statistical analysis; Leyla Soghandi: acquisition of data, critical revision of the manuscript for important intellectual content, administrative, technical, and material support. Reza Behmadi: analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and study supervision.

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References

1. Martin RJ, Fanaroff AA, Walsh MC. Fanaroff and Martin's neonatal-perinatal medicine: diseases of the fetus and infant. Elsevier Health Sciences; 2014.
2. Rennie JM, Roberton NRC. Textbook of neonatology. 1999
3. Clark RH, Bloom BT, Spitzer AR, Gerstmann DR. Empiric use of ampicillin and cefotaxime, compared with ampicillin and gentamicin, for neonates at risk for sepsis is associated with an increased risk of neonatal death. *Pediatrics.* 2006;**117**(1):67–74. doi: 10.1542/peds.2005-0179. [PubMed: 16396862].
4. Tullus K, Olsson-Liljequist B, Lundstrom G, Burman LG. Antibiotic Susceptibility of 629 Bacterial Blood and CSF Isolates from Swedish Infants and the Therapeutic Implications. *Acta Paediatrica.* 1991;**80**(2):205–12. doi: 10.1111/j.1651-2227.1991.tb11835.x.
5. Eidelman AI. Gentamicin-Resistant Klebsiella Infections in a Neonatal Intensive Care Unit. *JAMA.* 1978;**132**(4):421. doi: 10.1001/archpedi.1978.02120290093019.
6. Huang FY, Wang CH, Chiu NC. [Coagulase-negative staphylococcal septicemia]. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi.* 1994;**35**(5):423–8. [PubMed: 7942029].
7. Bromiker R, Arad I, Peleg O, Preminger A, Engelhard D. Neonatal Bacteremia: Patterns of Antibiotic Resistance. *Infect Control Hosp Epidemiol.* 2001;**22**(12):767–70. doi: 10.1086/501860.
8. Cowan ST, Barrow G, Steel KJ, Feltham R. Cowan and Steel's manual for the identification of medical bacteria. Cambridge university press; 2004.
9. Bauer A, Kirby W, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *J Clin Pathol.* 1966;**45**(4):493.
10. Clinical and Laboratory Standards Institute . CLSI/NCCLS M100-S152013. Performance standards for antimicrobial susceptibility testing, 5th informational supplement; .
11. Stoll BJ, Kliegman RM, Behrman RE, Jenson HB, Stanton BF. Nelson textbook of pediatrics. *Nelson textbook of pediatrics.* 2007.
12. Demissie S. Neonatal Sepsis: Bacterial Etiologic Agents And Their Antibiotic Susceptibility Pattern In Tikur Anbessa University Hospital, Addis Ababa, Ethiopia. aau; 2008.
13. Meremikwu MM, Nwachukwu CE, Asuquo AE, Okebe JU, Utsalo SJ. Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. *BMC Infect Dis.* 2005;**5**:110. doi: 10.1186/1471-2334-5-110. [PubMed: 16336657].
14. Rafati M, Farhadi R, Nemati-Hevelai E, Chabra A. Determination of Frequency and Antibiotic Resistance of Common Bacteria in Late Onset Sepsis at the Neonatal Ward in Booali-Sina Hospital of Sari, Iran. *JBMUS.* 2014;**16**(6):64–71.
15. Patel D, Nimbalkar A, Sethi A, Kungwani A, Nimbalkar S. Blood culture isolates in neonatal sepsis and their sensitivity in Anand District of India. *Indian J Pediatr.* 2014;**81**(8):785–90. doi: 10.1007/s12098-013-1314-2. [PubMed: 24408399].
16. Shehab El-Din EMR, El-Sokkary MMA, Bassiouny MR, Hassan R. Epidemiology of Neonatal Sepsis and Implicated Pathogens: A Study from Egypt. *BioMed res int.* 2015;**2015**.
17. Shah AJ, Mulla SA, Revdiwala SB. Neonatal sepsis: high antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit of a tertiary care hospital. *J Clin Neonatol.* 2012;**1**(2):72–5. doi: 10.4103/2249-4847.96753. [PubMed: 24027694].
18. Ballot DE, Nana T, Sriruttan C, Cooper PA. Bacterial bloodstream infections in neonates in a developing country. *ISRN Pediatr.* 2012;**2012**:508512. doi: 10.5402/2012/508512. [PubMed: 22919509].
19. Macones GA. Prematurity: Causes and Prevention. 2005 :139–45. doi: 10.1016/b978-072169347-7.50016-0.
20. Zaidi AK, Huskins W, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. *The Lancet.* 2005;**365**(9465):1175–88. doi: 10.1016/s0140-6736(05)71881-x.
21. Basiri B, Sabzehei MK, Shokouhi M, Moradi A. Evaluating the Incidence and Risk Factors of Nosocomial Infection in Neonates Hospitalized in the Neonatal Intensive Care Unit of Fatemieh Hospital in Hamadan, Iran, 2012 - 2013. *Pediatr Infect Dis J.* 2015;**3**(2) doi: 10.5812/pedinfect.23327.
22. Mosayebi Z, Movahedian AH, Soori T. Clinical and Bacteriological Characteristics of Neonatal Sepsis in an Intensive Care Unit in Kashan, Iran: A 2 Year Descriptive Study. *Pediatr Infect Dis J.* 2013;**1**(2):61–4. doi: 10.5812/pedinfect.7875.
23. Macharashvili N, Kourbatova E, Butsashvili M, Tsertsvadze T, McNutt LA, Leonard MK. Etiology of neonatal blood stream infections in Tbilisi, Republic of Georgia. *Int J Infect Dis.* 2009;**13**(4):499–505. doi: 10.1016/j.ijid.2008.08.020. [PubMed: 19058989].
24. Bhat YR, Lewis LE, K EV. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. *Ital J Pediatr.* 2011;**37**:32. doi: 10.1186/1824-7288-37-32. [PubMed: 21745376].
25. Bhutta ZA, Yusuf K. Early-onset neonatal sepsis in Pakistan: a case control study of risk factors in a birth cohort. *Am J Perinatol.* 1997;**14**(9):577–81. doi: 10.1055/s-2007-994338. [PubMed: 9394171].
26. Jurczak A, Kordek A, Grochans E, Giedrys-Kalemba S. Clinical forms of infections in neonates hospitalized in clinic of obstetrics and perinatology within the space of one year. *Advances in Medical Sciences (De Gruyter Open).* 2007;**52**.
27. Garcia FJ, Nager AL. Jaundice as an Early Diagnostic Sign of Urinary Tract Infection in Infancy. *Pediatrics.* 2002;**109**(5):846–51. doi: 10.1542/peds.109.5.846.
28. Youssef D, Elfateh H, Sedeek R, Seleem S. Epidemiology of urinary tract infection in neonatal intensive care unit: A single center study in Egypt. *J Am Acad Child Adolesc.* 2012;**2**(1):25. doi: 10.4103/2249-4855.104012.
29. Klein J, Long S. Bacterial infections of the urinary tract. 1995.
30. Movahedian AH, Mosayebi Z, Moniri R. Urinary tract infections in hospitalized newborns in Beheshti Hospital, Iran: A retrospective study. *J Infect Dis Antimicrob Agents.* 2007;**24**(1):7–11.

31. Musa-Aisien AS, Ibadin OM, Ukoh G, Akpede GO. Prevalence and antimicrobial sensitivity pattern in urinary tract infection in febrile under-5s at a children's emergency unit in Nigeria. *Ann Trop Paediatr.* 2003;**23**(1):39–45. doi: 10.1179/000349803125002850. [PubMed: 12648323].
32. Adeyemo AA, Gbadegesin RA, Onyemenem TN, Ekweozor CC. Urinary tract pathogens and antimicrobial sensitivity patterns in children in Ibadan, Nigeria. *Ann Trop Paediatr.* 1994;**14**(4):271–4. [PubMed: 7880087].
33. Das RN, Chandrashekhar TS, Joshi HS, Gurung M, Shrestha N, Shivananda PG. Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal. *Singapore Med J.* 2006;**47**(4):281–5. [PubMed: 16572238].
34. Musoke RN, Malenga GJ. Bacterial infections in neonates at the Kenyatta Hospital Nursery. A prospective study. *East Afr Med J.* 1984;**61**(12):909–16. [PubMed: 6536468].
35. Kasirye-Bainda E, Musoke FN. Neonatal morbidity and mortality at Kenyatta National Hospital newborn unit. *East Afr Med J.* 1992;**69**(7):360–5. [PubMed: 1396189].
36. Gaschignard J, Levy C, Romain O, Cohen R, Bingen E, Aujard Y, et al. Neonatal Bacterial Meningitis: 444 Cases in 7 Years. *Pediatr Infect Dis J.* 2011;**30**(3):212–7. [PubMed: 21416693].
37. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. To tap or not to tap: high likelihood of meningitis without sepsis among very low birth weight infants. *Pediatrics.* 2004;**113**(5):1181–6. [PubMed: 15121927].
38. Shattuck KE, Chonmaitree T. The Changing Spectrum of Neonatal Meningitis Over a Fifteen-Year Period. *Clinical Pediatrics.* 1992;**31**(3):130–6. doi: 10.1177/000992289203100301.
39. Isaacs D. The management of neonatal meningitis. *Current Paediatrics.* 2000;**10**(2):96–103. doi: 10.1054/cupe.2000.0082.
40. Garges HP, Moody MA, Cotten CM, Smith PB, Tiffany KF, Lenfestey R, et al. Neonatal meningitis: what is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters?. *Pediatrics.* 2006;**117**(4):1094–100. doi: 10.1542/peds.2005-1132. [PubMed: 16585303].