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Original Article

Positive Culture Samples of Infants with Neonatal Infections in a Tertiary Neonatal Center in Isfahan, Iran

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Abstract
Background: The prevalence of microorganisms in the neonatal intensive care unit (NICU) and neonatal internal wards is constantly changing, thus rendering the practice of empiric antibiotic therapy ineffective due to the resistance of these microorganisms. Therefore, the purpose of this study was to determine the relative frequency of positive cultures of Bactec, blood, cerebrospinal fluid (CSF) and urine in infants admitted to the NICU and neonatal internal ward in Al-Zahra hospital in 2011-2017.
Methods: In this cross-sectional descriptive study, we evaluated 466 positive culture samples from 2853 different cultures (blood, urine, CSF, etc) from infants admitted to the NICU and neonatal internal ward with clinical signs of neonatal infection in Al-Zahra hospital. Isfahan in 2011-2017. The samples were evaluated for type of microorganisms and sensitivity to antibiotics.
Results: Positive cultures among Bactec, blood, CSF and urine culture samples were reported at 15.5% (95% confidence interval [CI]: 12.8–18.1) 9.3% (95% CI: 6.8–11.7), 6.4% (95% CI: 4.3–8) and 28.6% (95% CI: 25.4–31.7), respectively. *Staphylococcus epidermidis* was the most common species in Bactec (46.7%; 95% CI: 38.7–54.6), blood (53.1%; 95% CI: 39.1–67), and CSF (37.1%; 95% CI: 21–53.1) cultures while *Klebsiella pneumoniae* was the most frequent species in urine culture (28%; 95% CI: 22.2–33.7).
Conclusion: Considering the results of Bactec and blood cultures, it is essential to reduce staphylococcal infections in our settings.
Keywords: Frequency, Microorganisms, Neonates, Sensitivity
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Introduction

Today, despite the advances made in the field of medicine, infections in the neonatal intensive care unit (NICU) remain one of the challenges of infection management. Furthermore, it is always desirable to reduce sensitivity to antibiotics in low birth weight or preterm infants.[1,2] Nosocomial infections are among common causes of infant mortality in NICUs, and risk factors such as preterm birth, weight less than 1500 grams, severe diseases such as meconium aspiration, prolonged NICU stay and low quality of care are associated with infection in these centers.

Among the risk factors mentioned, low birth weight and preterm birth are among the most important risk factors for infections.[3] In recent years, the causes of nosocomial infection have changed; since the 1970s, Group B Streptococcus (GBS) was the most common cause of early-onset sepsis (neonatal infection among those under 3-4 days of age), whereas today, *E. coli* and *Listeria monocytogenes* together with GBS constitute the most common causes of early-onset sepsis.[4]

Obtaining blood cultures, urine cultures and cerebrospinal fluid (CSF) cultures are routine and highly effective methods for diagnosis of infections, especially infections in the NICU. Using these techniques, definitive and effective treatment against the causative pathogen may be established. Preparing blood culture is one of the essential methods for diagnosis of bacterial infections (bacteremia) and fungal infections (fungemia) in the blood.[5]

Urinalysis is a standard diagnostic test for urinary tract infections (UTI) and is routinely implemented for patients with a diagnosis of UTI.[6] *E. coli* is one of the most common causes of UTI. It is responsible for 40%-72% of all UTI cases.[7] CSF culture is the gold standard (test) for diagnosis of bacterial meningitis.[8] Antibiogram is a summary of antibiotic susceptibility data for bacteria that is provided by the microbiological laboratory during a specific period (usually one year).

Physicians may use the antibiogram for empirical therapy. It can also be used in surveillance programs to improve treatment protocols and show the tendency

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toward microbial resistance.[9] Therefore, in this study, we aimed to detect different pathogens in the NICU by collecting antibiogram data for five consecutive years and then examine the sensitivity of each one to specific antibiotics in an attempt to improve the empirical therapy of these diseases and increase the efficacy of medications against the organisms to both achieve better and more efficient recovery, and reduce the costs for the health system of the country.

Materials and Methods

In this retrospective cross-sectional descriptive study, we obtained and evaluated 466 positive culture samples from 2,853 different culture samples (969 Bactec samples, 527 blood samples, 545 CSF samples and 812 urine samples) which were obtained from infants admitted to the NICU and the neonatal ward of Al-Zahra hospital, Isfahan in 2012–2017. In our center, infants are evaluated with culture samples if they have signs of neonatal infection such as poor feeding or poor sucking, hypotonia, decreased movement, abdominal distention, frequent nausea and vomiting, respiratory distress, cyanosis etc. The inclusion criteria were all culture samples from infants who had any signs/symptoms of neonatal sepsis and were admitted to the NICU or the neonatal ward.

In addition, incomplete culture reports were excluded from the study. In this study, considering the retrospective design, we extracted all of the cultures obtained from the admitted infants within the last five years using the data centers of Al-Zahra hospital information and computer center. Subsequently, we determined the number of positive blood cultures, urine cultures, and CSF cultures. Besides, in cases with positive samples, we specified the percentage of grown microbial strains in blood cultures, urinary cultures, and CSF cultures during these 5 years.

Then, based on the antibiogram of the positive samples, we determined the cases of antibiotic susceptibility to common strains in the neonatal wards. Data were analyzed using SPSS software version 22. The frequency and proportion of positive cultures were computed and 95% confidence intervals (CIs) were reported for computed proportions. Chi-square test was used to compare the frequency of positive cultures between different types of culture approaches. A *P* value less than 0.05 was considered as statistically significant.

Results

In this study, the frequencies of reportedly positive cultures were 179 (16.7%; 95% CI: 14.4–18.9) out of 1070 cultures from 2012 to 2013; 154 (17.2%; 95% CI: 14.7–19.6) out of 895 cultures from 2014 to 2015, and 133 (14.9%; 95% CI: 12.5–17.2) out of 889 cultures from 2016 to 2017. The frequency (prevalence) of positive cases for each type of culture on a yearly basis is summarized in Table 1.

Of the overall 466 positive cultures, 150 out of 969 Bactec cultures (15.5%; 95% CI: 12.8–18.1), 49 out of 527 blood cultures (9.3%; 95% CI: 6.8–11.7), 35 out of 545 CSF cultures (6.4%; 95% CI: 4.3–8) and 232 out of 812 urine cultures (28.6%; 95% CI: 25.4–31.7) were reported to be positive.

Among the Bactec cultures, the most common species were *Staphylococcus epidermidis* (46.7%; 95% CI: 38.7–54.6) and *Klebsiella pneumoniae* (14%; 95% CI: 8.5–19.5), *Enterococcus* (7.3%; 95% CI: 3.1–11.4) and *Acinetobacter* (6.7%; 95% CI: 2.6–10.7). Among the blood cultures, the most frequent species were *S. epidermidis* (53.1%; 95% CI: 39.1–67), followed by *Acinetobacter* spp., *Klebsiella*, and the fungi. The most common species in the CSF cultures were *S. epidermidis* (37.1%; 95% CI: 21–53.1) followed by *Klebsiella* and *Acinetobacter*. The most frequent species in urine cultures were *K. pneumoniae* (28%; 95% CI: 22.2–33.7), fungi (25.4%; 95% CI: 19.7–31), *Enterococcus* (14.7%; 95% CI: 110.1–19.2) and *E. coli* (6.9%; 95% CI: 3.6–10.1). Also, there was a significant difference between different cultures based on the type of species ($P < 0.001$). The types of other species based on the culture are summarized in Table 2.

Regarding the antibiotics sensitivity of common species, we found sensitivity to Vancomycin in 9 cases of *Staphylococcus aureus* (52.9%; 95% CI: 29.1–76.6), sensitivity to Clindamycin in 14 cases of *S. aureus* (82.4%; 95% CI: 64–100), sensitivity to Gentamicin in 2 cases of *S. aure*us (11.8%; 95% CI: 0–27.1) and sensitivity to Ceftazidime in 16 cases of *K. pneumoniae* (16.7%; 95% CI: 9.2–24.1), 7 cases of *Pseudomonas aeruginosa* (100%) and 5 cases of *Enterococcus* (17.6%; 95% CI: 7.6–27.5). We found sensitivity to Erythromycin in 9 cases of *S. aureus* (52.9%; 95% CI: 35.9–69.8), sensitivity to Cefepime in 18 cases of *K. pneumoniae* (18.8%; 95% CI: 10.9–26.6) and sensitivity to Co-trimoxazole in 14 cases of *K. pneumoniae* (14.6%; 95% CI: 7.5–21.6), 13 cases

Table 1. Frequency Distribution of Positive Cultures Based on Different Years and Sources

Year	Type of culture*			
	Bactec	Blood	CSF	Urine
2012-2013	66 (6.1%; 95% CI: 4.6–7.5)	20 (1.8%; 95% CI: 1–2.5)	14 (1.3%; 95% CI: 0.6– 1.9)	79 (7.3%; 95% CI: 5.7–8.8)
2014-2015	40 (4.4%; 95% CI: 3–5.7)	24 (2.6%; 95% CI: 1.5–3.6)	19 (2.1%; 95% CI: 1.1–3)	71 (7.9% ,95% CI: 6.1–9.6)
2016-2017	44 (4.9%; 95% CI: 3.4–6.3)	5 (0.5%; 95% CI: 0–9.6)	2 (0.2%; 95% CI: 0–0.4)	82 (9.2%; 95% CI: 7.3–11)

CSF, cerebrospinal fluid.

* The 95% confidence interval (CI) was used in reporting our research findings.

Table 2. Frequency of Different Microorganisms in Positive Culture Samples

Microorganisms	Type of Culture*			
	Bactec	Blood	CSF	Urine
Acinetobacter baumannii	2 (1.3%; 95% CI: 0–3.1)	0	4 (11.4%; 95% CI: 0.8–21.9)	2 (0.9%; 95% CI: 0–2.1)
Acinetobacter spp.	10 (6.7%; 95% CI: 2.6–10.7)	7 (14.3%; 95% CI: 4.4–24.1)	3 (8.6%; 95% CI: 0–17.8)	23 (10%; 95% CI: 6.1–13.8)
Enterobacter aerogenes	2 (1.3%; 95% CI: 0–3.1)	0	1 (2.9%; 95% CI: 0–8.4)	12 (5.2%; 95% CI: 2.3–8)
Enterococcus spp.	11 (7.3%; 95% CI: 3.1–11.4)	1 (2%; 95% CI: 0–5.9)	3 (8.6%; 95% CI: 0–17.8)	34 (14.7%; 95% CI: 10.1– 19.2)
Escherichia coli	3 (2%; 95% CI: 0–4.2)	1 (2%; 95% CI: 0–5.9)	1 (2.9%; 95% CI: 0–8.4)	16 (6.9%; 95% CI: 3.6–10.1)
Fungi	8 (5.3%; 95% CI: 1.7–.8)	3 (6.1%; 95% CI: 0–12.8)	1 (2.9%; 95% CI: 0–8.4)	59 (25.4%; 95% CI: 19.7–31)
Klebsiella pneumoniae	21 (14%; 95% CI: 8.5–19.5)	3 (6.1%; 95% CI: 0–12.8)	4 (11.4%; 95% CI: 0.8–21.9)	65 (28%; 95% CI: 22.2–32.7)
Pseudomonas aeruginosa	2 (1.3%; 95% CI: 0–3.1)	1 (2%; 95% CI: 0–5.9)	0	4 (1.7%; 95% CI: 0–3.3)
Staphylococcus aureus	7 (4.7%; 95% CI: 1.3–8)	0	3 (8.6%; 95% CI: 0–17.8)	2 (0.9%; 95% CI: 0–2.1)
Staphylococcus epidermidis	70 (46.7%; 95% CI: 38.7–54.6)	26 (53.1%; 95% CI: 39.1– 67)	13 (37.1%; 95% CI: 21–53.1)	14 (6%; 95% CI: 3.6–10.1)
Others	14 (9.3%; 95% CI: 4.6–13.9)	7 (14.3%; 95% CI: 4.4–24.1)	3 (8.6%; 95% CI: 0–17.8)	23 (10%; 95% CI: 6.1–13.8)
Total	150	49	35	232

CSF, cerebrospinal fluid.

* The 95% confidence interval (CI) was used in reporting our research findings.

of *S. aureus* (76.5%; 95% CI: 62.1–90.8) and 11 cases of *Acinetobacter* (52.4%; 95% CI: 37.4–67.3). We found sensitivity to oxacillin in 12 cases of *S. aureus* (70.6%; 95% CI: 55.1–86) and 9 cases of *K. pneumoniae* (9.4%; 95% CI: 3.5–15.2), susceptibility to ampicillin in 9 cases of *S. aureus* (52.9%; 95% CI: 37.4–67.3) and 7 cases of *Enterococcus* (13.7%; 95% CI: 6.9–20.4), sensitivity to Tazocin in 21 cases of *K. pneumoniae* 21.9%; 95% CI: 13.6–30.1), 5 cases of *E. coli* (22.7%; 95% CI: 10.1–35.2), sensitivity to amikacin in 27 cases of *K. pneumoniae* (28.1%; 95% CI: 19.1–37) and 15 cases of *E. coli* (68.2%; 95% CI: 54.2– 82.1), sensitivity to Mupirocin in 71 cases of *K. pneumoniae* (74%; 95% CI: 65.2–82.7), 19 cases of *E. coli* (86.4 %; 95% CI: 76.1–96.6)) And 14 cases of *Enterobacter aerogenes* (82.4%; 95% CI: 69.4–95.3), sensitivity to Cefotaxime in 13 cases of *K. pneumoniae* (13.5%; 95% CI: 6.6–20.3) and 7 cases of *E. coli* (31.8%; 95% CI: 17.8–45.7), susceptibility to nitrofurantoin in 12 cases of *Enterococcus* (23.5%; 95% CI: 15.1–31.8) and 8 cases of *E. coli* 36.4%; 95% CI: 22–50.7). We also observed sensitivity to linezolid in 25 cases of *Enterococcus* (49%; 95% CI: 39.2–58.8), sensitivity to colistin in 16 cases of *K. pneumoniae* (16.7%; 95% CI: 3.6–10.1) and 8 cases of *A. baumannii* (100%) and sensitivity to levofloxacin in 12 cases of *K. pneumoniae* (12.5%; 95% CI: 7.7–19.1).

Discussion

The most common species in the total number of culture samples in the NICU and the neonatal ward of Al-Zahra hospital were *S. epidermidis*, *K. pneumoniae*, fungi, *Enterococcus*, *E. coli*, and *Acinetobacter*, in decreasing order of frequency. In our study, the most common species in the blood and urine sample were *S. epidermis* and *Klebsiella*, respectively.

In the same vein, a study by Villari et al reported that negative coagulase staphylococci, especially *S. epidermidis*, are one of the most common (bacterial) strains in hospitals, especially in neonatal wards. In this study, which evaluated the nosocomial infections in NICU during three years, it is suggested that *S. epidermidis* is one of the major causes of infections in the NICU, which reduces the sensitivity to glycopeptides, and this decrease in glycopeptide sensitivity increases the resistance of these strains to most antibiotics.[10] In fact, according to this study, the most common bacterial type in the wards is *S. epidermis*, which exists as normal skin flora and contamination.

Also, Behmadi et al[11] examined the most common species in the CSF, urine, and blood culture samples obtained from patients in the NICU and neonatal ward of Hakim hospital in Neyshabur, Iran. They concluded that negative coagulase staphylococci were the most common pathogens in the blood samples at the early-onset and late-onset cases. In addition, *E. coli* and *Klebsiella* were the most common species in early-onset and late-onset UTI.

It should be noted that in this study, these species were highly resistant to antibiotics that had been prescribed empirically and that the frequency (prevalence) of meningitis was low.[11] In our study, the most common species in the blood samples were *S. epidermidis*, followed by *Acinetobacter* spp. and the most common species in the urine cultures were *K. pneumoniae*, fungi, *Enterococcus* and finally, *E. coli*.

In our study, the most common strains which were present in meningitis were *S. epidermidis* followed by *Klebsiella*. In other studies, common species in the meningitis in patients under three days of age were similar to neonatal early-onset sepsis and included GBS or *Streptococcus agalactiae*, *E. coli*, and *Listeria monocytogenes*,[8,12] whereas late-onset meningitis was caused by GBS, *E. coli*, *Listeria monocytogenes*, gram-negative bacteria, staphylococci and *Streptococcus pneumoniae*.[13] The high frequency (prevalence) of *S. epidermidis* in our study might have resulted from non-sterile lumbar puncture (LP).

In a study by Gupta, *K. pneumoniae* was one of the most important species in the hospital that caused septicemia, infection, urinary tract infection, pneumonia and soft tissue infection, especially in newborns. In this study, it was stated that due to the high frequency of this microorganism in neonate patients, multiple drug resistance has increased in these strains, and sensitivity tests usually reveal limited therapeutic options. Recently, as a result of increased antibiotic resistance, mortality due to this microorganism has increased, and use of 3rd generation cephalosporins has had an effective role in reducing mortality.[14]

One of the most prominent features of this study is that we evaluated Bactec, blood, CSF and urine cultures obtained from the patients admitted to the NICU and neonatal ward of Al-Zahra hospital over a 5-year period. On the other hand, the most significant limitations of our study include the large number of positive cultures, the unavailability of the patient's age for evaluating early-onset and late-onset infections, lack of information to ensure sterilization of sampling methods, the single-centered nature and the relationship between the final outcomes and clinical findings, positive culture samples and *antibiotic therapy*.

Sterile sampling is a routine provision in neonatal wards; nevertheless, similar to other wards, it is not possible in practice to separate contaminated samples from the truly positive cases. However, we limited the cultures to the samples of the neonates with signs of neonatal infection.

Also, the percentage of positive CSF and blood cultures was low compared to the total number of samples in this study. It is possible that a percentage of positive cultures are not included due to technical laboratory analysis errors and the results probably do not perfectly represent the population of hospitalized neonates.

By carefully evaluating and planning, we can identify common types of microorganisms in NICUs and neonatal wards and choose the appropriate antibiotic for treatment.

Given that the prevalence of species which exist in NICUs and neonatal wards changes gradually, as well as the fact that sensitivity and resistance to antibiotics are subject to change, it is necessary to conduct intermittent epidemiologic studies at specific intervals to determine the frequency of common bacterial species and antibiotic sensitivity.

Authors' Contribution

All stages of design, performance and writing of the study were performed with AMA, MF and NS.

Conflict of Interest Disclosures

There are no conflicts of interest.

Ethical Statement

This study is taken from the doctoral thesis of Dr. Farajollahi. All stages of the design and implementation of the current study were approved by the Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran.

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