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Original Article

# Determination of Antibiotic Resistance Pattern of Bacteria Isolated from Blood, Cerebrospinal Fluid, and Urine Samples in Neonatal Intensive Care Unit of Ali Asghar Hospital, Iran during 2013-15

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## ABSTRACT

***Background:*** Infections are one of the most important causes of death in infants, especially in developing countries. Inappropriate use and administration of antibiotics can contribute to the resistance and spread of infection. In this study, we determined the antibiotic resistance pattern of the bacteria isolated from clinical samples of blood, cerebrospinal fluid, and urine in the neonatal intensive care unit (NICU) of Ali Asghar Hospital, Iran during 2013-2015.
***Methods:*** For the purposes of the study, clinical samples of blood, cerebrospinal fluid, and urine were collected from the NICU of Ali Asghar Hospital during 2013-15. The type of bacterial strain and antibiotic susceptibility pattern was determined by routine microbiological tests. The collected data were analysed in SPSS software (version 19), using $\chi 2$, Student's t-test, and ANOVA test for comparison.
***Results:*** In total, 240 positive culture samples (118 blood, 117 urine, and 5 cerebrospinal fluid samples) were collected for this research. The most common isolates in the blood, urine, and cerebrospinal fluid samples were *Staphylococcus epidermidis* (63.6%), *Klebsiella* (35%), and *Acinetobacter Baumannii* (40%), respectively. The highest antibiotic resistance in *S. epidermidis* isolated from blood samples was found against amikacin and cefotaxime, while linezolid and vancomycin were the most effective antibiotics against S. *epidermidis*. Moreover, ciprofloxacin and nitrofurantoin were the most effective antibiotics against *Klebsiella* isolated from urine samples, while this bacterial strain had the highest resistance to imipenem and ampicillin. Despite the fact that *A. Baumannii* strains were resistant to most studied antibiotics, ceftazidime and ceftriaxone had an acceptable antibacterial effect against these isolates.
***Conclusion:*** Continuous surveillance for antibiotic susceptibility, rational use of antibiotics, and the strategy of antibiotic cycling can provide some answers to the emerging problem of antibiotic resistance.

***Keywords:*** Antibiotic resistance, Infection, Neonates, NICU

## Introduction

Bacterial infections are one of the most important mortality factors during the neonatal period in neonatal intensive care units (NICU) throughout the world. Anti-bacterial treatment in infants and children usually faces numerous challenges. In different age groups, there are clear differences among pathogen species which are responsible for bacterial infection. In addition, selecting the dosage and interval of antibiotics is related to the age of the patient and its toxicity depends on the physiology of each infant's body (1, 2). An appropriate and specific antibiotic therapy depends on specific microbiological diagnosis tests, especially antimicrobial susceptibility tests following bacterial isolation from a sterile part of the body, such as blood urine and cerebrospinal

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fluid (CSF) (3, 4). However, because of serious problems in clinical sample collection, immune system deficiency, non-specific signs of diseases, and subsequently increased risk of death in newborns, antibiotic therapy is immediately performed based on initial clinical diagnosis to prevent wasting time (5, 6). Therefore, it is necessary to obtain appropriate information about the most common type of probable pathogens in NICU, as well as their sensitivity or resistance to a wide range of antibiotics to choose the most appropriate ones (7).

By the advent of new resistance mechanisms and overuse of antibiotics in health care department, medicine has practically confronted with some incurable bacterial infections, especially at NICU. As a result, we need a new generation of antibacterial agents. Regarding the mentioned problems, application of antibiotics with narrowing antimicrobial spectrum should be limited to those cases that are really necessary which prevents microbial resistance (8). Therefore, constant epidemiological monitoring is very important as there are numerous reports based on the vast prevalence of antibiotic resistance in different parts of hospitals throughout the world.

In this research, we considered the sensitivity and antibiotic resistance of bacteria isolated from blood, CSF, and urine of newborns hospitalized in the NICU at Ali-Asghar hospital. We hope that the obtained results can provide proper and valuable information for specialists in order to prohibit the spread of more antibiotic resistance isolates in the NICU and prevent mortality among neonates.

## Methods

### *Sample collection*

In this cross-sectional study, all the information of newborns with positive blood, urine, or CSF culture results who were hospitalized in NICU at Ali-Asghar hospital during 2013-2015 were extracted from the hospital information system. Demographic data of the newborns such as age, gender, weight, height, type of birth, and other necessary information were recorded as well. Only newborns with positive blood, urine, or CSF culture results were enrolled in this study. Therefore, those with the age of more than 28 days or negative culture results were excluded. After the selection process based on inclusion and exclusion criteria, a total of 240 positive culture samples (118 blood, 117 urine, and 5 CSF samples) that was noted for each newborn were collected. Type of microbial strains was also recorded based on the standard biochemical and microbiological tests (e.g. gram stein, catalase, oxidase, lactose, sucrose, chocolate agar and MacConkey culture medium, catalase test, coagulase test, sensitivity to Novobiocin disc). Furthermore, the results of the antibiotic susceptibility test (antibiogram) were obtained which was performed based on the disc distribution method and principles of clinical and laboratory standard institute.

### *Statistical analysis*

Descriptive statistics were applied for the analysis of frequencies among the patient groups. Furthermore, the Chi-square test was applied to compare the qualitative variables between the groups, while the ANOVA test and t-test were used to compare quantitative variables between them. The data were analyzed in SPSS software (version 19) and P< 0.05 was considered statistically significant.

## Results

In this study, a total of 240 clinical samples were collected. Most of the isolates were provided from the blood (118) and urine (117) samples, while only 5 isolates were collected from the CSF samples. The mean age of all the participating neonates was 12.12±8.45 days (ranging from 1-29 days) and 54.6% (131) of them were male. There was no significant difference between the mean ages of the male (12.42±8.89 days) and female (11.76±7.92 days) neonates (P=0.54). The distribution frequency of males and females, as well as the mean ages of neonates in each clinical sample group, is summarized in Table 1. A significant difference was found between the mean ages of neonates in each clinical sample group (P<0.001). The mean age of neonates in CSF clinical samples was significantly higher than that in blood and urine sample groups.

The frequency of each bacterial strain in all the clinical samples is shown in Table 2.

**Table 1.** Distribution of clinical samples based on gender

| | Blood sample (n=118) | Urine sample (n=117) | Cerebrospinal fluid sample (n=5) | p-value |
|---|---|---|---|---|
| Age (day) | 9.88±8.9 | 13.96±7.21 | 21.8±9.09 | <0.0001 |
| Gender | | | | |
| Male (%) | 69 (57.6%) | 59 (50.4%) | 3 (60%) | 0.45 |
| Female (%) | 49 (42.4%) | 58 (49.6%) | 2 (40.0%) | |

**Table 2.** Distribution of bacterial isolates in the clinical samples

| Isolates | Frequency | Valid Percent |
|---|---|---|
| *Staphylococcus epidermidis* | 79 | 32.9% |
| *Klebsiella* | 49 | 20.4% |
| *E. coli* | 29 | 12.1% |
| *Non-albicans Candida* | 23 | 9.6% |
| *Enterococcus* | 12 | 5.0% |
| *Staph haemolyticus* | 11 | 4.6% |
| *Acinetobacter Baumannii* | 7 | 2.9% |
| *Staph Saprophyticus* | 6 | 2.5% |
| *Candida Albicans* | 5 | 2.1% |
| *Staph. Aureus* | 5 | 2.1% |
| *Enterobacter* | 5 | 2.1% |
| *Group D streptococci* | 1 | 0.4% |
| *Streptococci* non-groups *A, B, or D* | 1 | 0.4% |
| *Pseudomonas aeroginosa* | 1 | 0.4% |
| *Pneumococci* | 1 | 0.4% |
| *Viridans streptococci* | 1 | 0.4% |
| *Micrococci* | 1 | 0.4% |
| *Alcaligenes Spp* | 1 | 0.4% |
| *Gram negative Bacilli* | 1 | 0.4% |
| *Streptococcus pneumoniae* | 1 | 0.4% |

Approximately 20 bacterial strains were isolated from clinical samples in NICUI. *Staphylococcus epidermidis* (32.9%), *Klebsiella* (20.4%), and *E. coli* (12.1%) were the most common types of bacterial strains isolated from clinical samples. The frequency of each bacterial strain in each clinical sample is shown in Table 3. The most common organisms in the blood, urine, and CSF samples were *S. epidermidis* (63.6%), *Klebsiella pneumonia* (35%), and *Acinetobacter baumannii* (40%), respectively.

Further analysis showed that the highest antibiotic resistance in *S. epidermidis* isolated from the blood samples was related to amikacin (100%) and cefotaxime (100%), while the most sensitivity was found for linezolid (98.7%) and Vancomycin (97.3%). Despite the fact that the isolated *Klebsiella* from urine samples was resistant to ampicillin (97.4%), imipenem (100%), and cefazolin (82.9%) antibiotics, ciprofloxacin, nitrofurantoin, and Co-trimoxazole were the most effective antibiotics against this isolate showing 82.5%, 76.9%, and 62.5% sensitivity, respectively. The *A. baumannii* isolated from CSF samples showed high resistance against most antibiotics; however, ceftazidime, ceftriaxone, and ciprofloxacin were the most effective antibiotics against this isolate showing 50%, 50%, and 50% sensitivity, respectively.

**Table 3.** Distribution of bacterial isolates in each clinical sample

| Bacterial isolates | Samples | | |
|---|---|---|---|
| | Blood | Urine | Cerebrospinal fluid |
| *Staphylococcus epidermidis* | 75 | 4 | 0 |
| *Klebsiella* | 8 | 41 | 0 |
| *E. coli* | 4 | 25 | 0 |
| *Non-albicans Candida* | 0 | 22 | 1 |
| *Enterococcus* | 1 | 11 | 0 |
| *Staph haemolyticus* | 7 | 3 | 1 |
| *Acinetobacter Baumannii* | 4 | 1 | 2 |
| *Staph Saprophyticus* | 5 | 1 | 0 |
| *Candida albicans* | 0 | 5 | 0 |
| *Staph. Aureus* | 5 | 0 | 0 |
| *Enterobacter* | 3 | 2 | 0 |
| *Group D streptococci* | 0 | 1 | 0 |
| *Streptococci* non-groups *A, B, or D* | 1 | 0 | 0 |
| *Pseudomonas aeroginosa* | 0 | 1 | 0 |
| *Pneumococci* | 0 | 0 | 1 |
| *Viridans streptococci* | 1 | 0 | 0 |
| *Micrococci* | 1 | 0 | 0 |
| *Alcaligenes Spp* | 1 | 0 | 0 |
| *Gram negative Bacilli* | 1 | 0 | 0 |
| *Streptococcus pneumoniae* | 1 | 0 | 0 |

## Discussion

The bacterial infection is one of the most important mortality reasons in newborns in NICUs. Due to innate immune deficiency and the outbreak of nonspecific signs for infections in different organs of the body, newborns have a higher risk of death (1, 2). Therefore, proper antibiotic therapy is essential following possible differential diagnoses based on physical examinations, history, and primary laboratory evidence, as well as the possible diagnosis of common pathogens before culture. Recent studies have demonstrated that there has been an increase in antibiotic resistance patterns in intensive care units, especially in NICU and PICU.

Therefore, novel broad-spectrum and more effective antibiotics are needed against multi-resistant strains.

Choosing an appropriate antibiotic is the priority of each country and each hospital. It reduces the incidence of new resistance organisms and avoids wasting time and budget in order to produce newer drugs (9). The present research aimed to consider the incidence of bacterial strains and their antibiotic resistance pattern from blood, CSF, and urine samples of newborns hospitalized in the NICU of Ali Asghar Hospital. As it is known, vancomycin and linezolid were the most effective antibiotics against *S. epidermidis*. The results of this study did not reveal a significant difference between the antibacterial activity of vancomycin (97.3%) and linezolid (98.7%) against this strain. In this circumstance, the selection of vancomycin in the first step has the priority to linezolid in order to prevent the spread of new antibiotic resistance isolates. This data suggests that linezolid should be saved as the strongest drug and not be overused. We also found that the maximum antibiotic sensitivity of *Klebsiella* isolate in urine samples was related to ciprofloxacin, nitrofurantoin, and Co-trimoxazole. However, nitrofurantoin must not be used in neonatal urinary tract infections since it does not affect the treatment of pyelonephritis and is just used for lower urinary tract infections. It is also notable that *klebsiella* had low sensitivity to third and fourth generation cephalosporins (10). Therefore, this data suggests that ciprofloxacin should be used as the first line of antibiotic therapy for UTI due to the high incidence rate of *Klebsiella*. Co-trimoxazole is very effective; however, its consumption is forbidden for newborns since they increase the risk of kernicterus (11). Moreover, the use of ciprofloxacin is not recommended for children under 18 years old. For these reasons, the treatment of *klebsiella* requires combination therapy in most cases.

In this research, *A. baumannii* that was isolated from CSF samples showed high resistance to most antibiotics. Nevertheless, ceftazidime, ceftriaxone, and ciprofloxacin showed acceptable antibacterial effect against this strain. Regarding the small number of CSF clinical samples (n=5), the obtained results are not significant and so further studies should be performed with larger sample sizes. The *A. baumannii* is a Gram-negative bacillus that is aerobic, pleomorphic, and non-motile. An opportunistic pathogen, *A. baumannii* is an important opportunistic and emerging pathogen that can lead to serious nosocomial infections. Its pathogenic potential includes the ability to adhere to surfaces, form biofilms, display antimicrobial resistance, and acquire genetic material from unrelated genera. All of these features make it a versatile and difficult adversary to control and eliminate. The optimal treatment has been established for *A. baumannii*, especially nosocomial infections resulting from multiple resistant strains (12).

Unfortunately, the laboratory of our hospital has not used Colistin disk in recent years. Therefore, specification of the sensitivity and microbial resistance to *A. baumannii,* and thereby determination of the microbial sensitivity and resistance of this organism to colistin was not possible in this time period. Shrestk *et al*., isolated pathogens from clinical samples of blood, urine, eye swabs, and feces of newborns. They found that coagulase-negative *staphylococci* and *Acinetobacter* were the most common types of isolated bacteria from the blood (13). This finding is in accordance with the results of the present study. More than 30% of Gram-negative isolates were cefotaxime-resistant and more than 50% of them were gentamycin-resistant. This indicates the resistance of a considerable percentage of these isolates to the first line of antibiotic therapies. Rajabi *et al*. conducted a study on 115 positive blood samples, in which 95% of isolates were Gram-negative (*Klebsiella* and *Enterobacter*) and 50% of isolates were Gram-positive (*S. epidermidis*). In the above-mentioned study, ciprofloxacin was the most effective antibiotic against Gram-negative bacteria, while vancomycin (80%) was found to be more effective against Gram-positive strains (14). Similarly, Fahmey (15) screened the prevalence of bacterial isolates and antibiotic resistance patterns in 673 neonates at the NICU. They found that 138 (20.5%) blood cultures were positive. Approximately, 86.2% of isolates were Gram-negative pathogens. *Klebsiella pneumoniae* (42.8%), *Enterobacter cloacae* (22.5%), and *Escherichia coli* (13.8%) were the most common types of isolated gram-negative bacteria. *Staphylococcus aureus* was the commonest type of isolated Gram-positive. They also reported that *Klebsiella* (100%) and Enterobacter (93%) isolates were resistant to ampicillin. Gram-negative bacteria had the maximum overall sensitivity to ciprofloxacin, cefepime, and imipenem, while vancomycin, imipenem, and piperacillin were the most effective antibiotics against Gram-positive isolates. In another research, Sorsa *et al*., found that 29.4% of blood cultures were positive for neonates hospitalized in the NICU and among these, Coagulase-negative *staphylococci* (25%), *Escherichia coli* (20.5%), and *Staphylococcus aureus* (18%) were the most common type of isolates in the blood culture. Resistance rates for coagulase-negative *staphylococci* and *Staphylococcus aureus* against ampicillin were 91% and 69% respectively. Ampicillin and gentamycin were the most effective antibiotics against *E. coli*, as resistance rate of 66.7% and 55.6%, in order. indeed, *Klebsiella* was more resistant against Ampicillin and Gentamycin antibiotics, showing a resistant rate of 91% and 82%, respectively. (16)

According to the accomplished data from previous studies and results from this study, the results obtained from such epidemic studies cannot be generalized to all hospitals and health centers given the various amount and the type of selected antibiotics in each center. This will result in different bacterial resistance mechanisms in each center and consequently, pathogens may have various antibiograms. antibiotic susceptibility through MCI method is more efficient in comparison to pathogens isolated from the patients' samples in different time periods and in various health centers. Furthermore, the use of the results of previous reports will be generally helpful in the selection of appropriate antibiotics for each organism.

## Conclusion

According to the results, *S. epidermidis*, *Klebsiella pneumonia,* and *A. baumannii* were the most common types of bacterial strains isolated from clinical samples of the blood, urine, and CSF samples, respectively, in neonates who were hospitalized in NICU. Therefore, the incidence rate of bacterial infection and their antibiotic resistance pattern should be determined in each hospital annually to prevent mortality in neonates hospitalized in NICU. Further studies are essential to the development of strategies for the management of antibiotic resistance in these isolates in the NICU.

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## Conflicts of interest

The authors have no conflicts of interest.

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