Etiological agents of infections in neonatal intensive care units

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ABSTRACT
Objectives: Neonatal or newborn period includes the first 28 days after the birth of a child. The immune system of a newborn is not fully developed and can not be completely effective in the period just before birth and seven days after. The aim of this study was to identify the most common causative agents of neonatal infections and the movement of infections through the observed period.

Material and methods: This retrospective analytical study recruited 160 patients admitted to the Neonatology Department of the Cantonal Hospital „Dr Irfan Ljubijankic“ in Bihac, in the period from January 2013 to December 2014 on suspicion of neonatal infection.

Results: Of 160 neonates suspected on neonatal infections in 123 (76,9%) was confirmed. Most neonates were admitted in the period from October to December 2013 (n=31, 19,4%). Most neonates with confirmed neonatal infection were admitted in the period from January to March 2013 (n=25, 20,3%). The most common diagnosis was Infectio perinatalis (n=57, 28,5%). Of the 87 isolated pathogens 65% (n=57) were Gram-positive, from which the most common were Staphylococcus aureus and MRSA 82,5% (n=47).

Conclusion: Of the total number of neonates admitted on suspicion of neonatal infection, healing was result in 75% (n=120), neurological deficit in 18,1% (n=29), and the death in 6,9% (n=11) neonates.

Keywords: neonates, Infectio perinatalis, Staphylococcus aureus, neurological deficit

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INTRODUCTION
Neonatal or newborn period includes the first 28 days after the birth of a child, so-called postnatal life. Although the development of specific immune system begins early in the intrauterine period, the immune system of the newborn is not fully developed and can not oppose pathogens which can be directly exposed before birth to seven days after. A newborn is in the first days of extraterine life protected with IgG antibodies which are maternal origin. They are transplacentally transferred mostly in the last trimester of pregnancy. Antibodies IgM and IgA do not cross the placenta, but a large amount of secretory IgA antibodies newborn is received by colostrum [1]. Neonatal infections are infections that happened during the first 28 days of life. The fetus is protected against bacterial infection, but after birth it comes to the colonization and the establishment of normal microflora. Some bacteria can lead to infection of the placenta and fetus infection in utero or to the development of neonatal infection during childbirth. Serious /acute/ neonatal infections include sepsis, meningitis, pneumonia and urinary tract infection. There are also possible focal bacterial infections (skin, navel and conjunctiva) and fungal, viral and protozoal infections /candidiasis, TORCH/ [2, 3]. Neonatal sepsis goes below a picture of bacteremia and systemic infection, and despite good antimicrobial therapy remains a major cause of morbidity and high infant mortality. The most common bacterial pathogens are β hemolytic Streptococcus group B (Streptococcus agalactiae), coagulate-negative Staphylococcus (CONS), Staphylococcus aureus, Escherichia coli, Haemophilus influenzae, Listeria monocytogenes, Klebsiella, Pseudomonas, Enterobacter, Serratia, Acinetobacter and anaerobic bacteria [4]. Newborn, and particularly those born prematurely, has sepsis often with has purulent meningitis. Clinical symptoms are non-specific and are characterized by an unstable temperature, drowsiness, abnormal or so called cerebral cry, apnea, seizures,
vomiting, tension fontanelle, increase head circumference. The most common cause is a β hemolytic streptococcus group B (Streptococcus agalactiae). Other causes include Listeria monocytogenes, Enterococcus spp, E. coli and other gram-negative bacteria (Klebsiella, Enterobacter, Serratia) and Staphylococcus aureus (particularly MRSA) and CoNS [5, 6]. Neonatal pneumonia is a lung infection of the newborn. If it occurs within a few hours of birth of a child, it is considered a part of generalized sepsis syndrome. Pneumonia with late onset occurs after seven days of birth. Etiological agents come from the mother’s genital tract or hospital room. Those are Gram-positive cocci (Streptococcus A and B, and Staphylococcus aureus) or Gram-negative bacilli (Escherichia coli, Klebsiella spp., Proteus spp.). The most common cause is E. coli, but causes are also and other Gram negative bacteria (Klebsiella pneumoniae, Enterococci etc.). [7, 8, 9, 10]. The incidence of urinary infections varies with birth weight and it is less than 3%. Bacteriuria can indicate the presence of generalized sepsis with hematogenous spread of the kidneys, but it can also be a primary kidney infection leading to a systemic infection [11, 12, 13]. The infected infants who are hospitalized in the Neonatal Intensive Care Division are population with the highest risk of neonatal hospital infections which are significant cause of their morbidity and mortality [14].

**MATERIALS AND METHODS**

Between February - April 2015, data was collected from the Department of Neonatology of the Cantonal Hospital Dr. Irfan Ljubijankic in Bihac from all patients of Una-Sana Canton who were treated at the Department of Neonatology in a period of two years (from 01.01.2013. till 31.12.2014). Samples were swabs of the the nose, nasopharynx, eye and ear, aspirates and blood.

In the study, isolation and identification of pathogens were performed routinely, taken over several days. In the laboratory, samples were tested using standard methods of diagnostics. The swabs were immediately seeded on blood agar, Mac Conkey agar and dextrose agar, then incubated for 24h at 37°C. When it was necessary, we approached to the biochemical and serological tests. After isolation and identification of bacteria, we tested the resistance of bacteria to antibacterial drugs. That test was done using disk diffusion method, according to Kirby-Bauer on Muller-Hinton agar by CLSI standards (Clinical and Laboratory Standards Institute). This method is one of the oldest approaches to antimicrobial susceptibility testing, based on the principle of diffusion of antibiotics/chemotherapeutics in the disc or tablet environment and growth inhibition of inoculated bacteria.

![Graph showing trend of receiving neonates admitted for suspected neonatal infection in the period January 2013 to December 2014.](image-url)
The obtained data were statistically analyzed using Excel and SPSS software version 19. Results are presented as absolute numbers (N) and percentage values (%). For nominal and ordinal variables it was used Chi-square test. The accepted level of statistical significance in the our study was \( p < 0.05 \). Results are presented in tables and graphs.

**Results**

In the period from January 2013 to December 2014 in the Cantonal Hospital Dr. Irfan Ljubijankic in Bihać, were recorded 3543 births. During this period, the Department of Neonatology, received 948 neonates (26.8% of total births). Number of neonates who were admitted for suspected neonatal infection, was 160 (16.9% of total received neonates).

From Table 1, it can be seen that total patients number is 160, from that male 96 (60%). Average birth weight was 3350 grams (range 2547.5 – 3777.5). Birth weight less than 2,500 grams was observed in 39 neonates. The number of received neonates born with less than 37 weeks gestation, was 32 (20%). Average age of neonates was 2 days (range 0-13). The mean age at the time of the onset of symptoms is 4 days.

From Figure 1, it is seen that the number of neonates who were admitted for suspected neonatal infection and the number of those with microbiologically confirmed diagnosis after the first quarter of 2013 years, began to collapse. In that first quarter was the largest number of patients with the confirmed diagnosis (n=25, 20.30%). Then, trend receipt was evidently fell to the third quarter of 2013 when it received (n=12, 7.5%) neonates, and of whom in 9 (7.3%) diagnosis was confirmed. After that, trend receipt was increased and in the last quarter of 2013 achieves its maximum value (n=31, 19.4%). In the first quarter of 2014 years continues to decline trend receipt of neonates to the second quarter had stagnation. In the last two quarters of the year, the trend reception neonates decreases.

Figure 2. shows that in the first quarter of 2013 the number of diagnosed neonates large (n=36; 18%). In this period of monitoring, diagnosis *Infectio perinatalis*...
(n=13, 22.8%) and *Conjunctivitis* (n=14; 30.4%), and reach their maximum values and account for 75% of the total number of diagnoses in the quarter. In the second and third quarter of the same year, the number of diagnosed neonates is reduced to the last quarter of 2013, achieved its highest value (n=38; 19%). During this period, diagnosis *Infectio tractus respiratorii* reaches its maximum value (n=14; 29.2%). With the beginning of 2014, the number of diagnosed neonates admitted for suspected neonatal infection, begins to decline and this trend is maintained until the end follow-up period.

**Table 2. Cause of neonatal infection and outcome.**

<table>
<thead>
<tr>
<th>Causes</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25</td>
<td>28.7</td>
</tr>
<tr>
<td>MRSA</td>
<td>22</td>
<td>25.3</td>
</tr>
<tr>
<td>Coagulase-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em> (CoNS)</td>
<td>3</td>
<td>3.4</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>4</td>
<td>4.6</td>
</tr>
<tr>
<td><em>Streptococcus β</em></td>
<td>3</td>
<td>3.7</td>
</tr>
<tr>
<td>hemolyticus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>16</td>
<td>18.4</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>5</td>
<td>5.7</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>5</td>
<td>5.7</td>
</tr>
<tr>
<td><em>Moraxella Catarrhalis</em></td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Haemophilus</em> spp.</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>2</td>
<td>2.3</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery</td>
<td>120</td>
<td>75</td>
</tr>
<tr>
<td>Neurologic disorders</td>
<td>29</td>
<td>18.1</td>
</tr>
<tr>
<td>Death</td>
<td>11</td>
<td>6.9</td>
</tr>
</tbody>
</table>

From Table 2, it can be seen that the most common cause of neonatal infection was *Staphylococcus aureus*, 28.7% (n=25) of the total number of causes of neonatal infections. The methicillin-resistant forms *Staphylococcus aureus* (MRSA) was the cause of 25.3% (n=22), while the CoNS (coagulase negative *Staphylococcus*) was the cause of 3.4% (n=3) cases. *Escherichia coli* was detected as the causative agent in 18.4% (n=16) of cases. Other pathogens were *Pseudomonas aeruginosa* 5.7% (n=5), *Klebsiella pneumoniae* 5.7% (n=5), *Enterococcus* spp. 4.6% (n=4), *Moraxella catarrhalis* 1.1% (n=1), *Haemophilus* spp. 1.1% (n=1), *Proteus vulgaris* 2.3% (n=2) and *Streptococcus β* hemolyticus 3.7% (n=3).

Healing was the outcome in 120 (75% of the total number of neonates admitted for suspected neonatal infection), a neurological deficit 29 patients (18.1%). Number of neonates admitted for suspected neonatal infection who died was 11 (6.9%).

**Discussion**

Bacterial infections of newborns are still growing medical problem. This study on the causes of neonatal infections in the Una-Sana Canton, according to our knowledge, is the first comprehensive review of neonatal infections and their causes in the Canton for the period from January 2013 to December 2014. Number of neonates who were admitted for suspected neonatal infection, was 160 (16.9% of total received neonates). From 160 neonates, most of them were received in the period from October to December 2013 (19.4%, n=31). From 123 neonates with confirmed neonatal infections, the most received ones were in the period from January to March 2013 (20.3%, n=25), and the most common diagnosed was *Infectio perinatalis* (28.5%, n=57). Of the 87 isolated microbial pathogens, 65.5% (n=57) were Gram-positive and 34.5% (n=30) Gram-negative.

Similar to our results, were the results of the study supervision of intra-hospital infections conducted in Italian neonatal intensive care units [15], where the highest number of diagnosis *Sepsis* accounted for 44.4%, followed by *Infectio tractus urinaria* 28.8%, *Pneumonia* 25, 5% and *Meningitis* 1,3% of the total number of diagnosed infections during the study.

These findings are similar to ours. In a study conducted surveillance of neonatal infections in England [16], the results showed that neonatal infection usually occurs in children born prematurely and/or with low birth weight. This can be related to the outcome of infection, which in our study, although it was the same distribution, had a different percentage values. So the percentage of death in our study was much higher in children born prematurely and/or with low birth weight, while the percentage of recovery was much higher among children born in the time and/or with a birth weight greater than 2,500 grams. In the English study, the incidence of wound sepsis was lower than later and its causes are most frequently Streptococcus group B (58% of the total number of agents that caused the wound sepsis) and *Escherichia coli* (18%). Later, the most common cause were: CONS (54% of the total number of agents that caused the late sepsis), *Enterobacteriacae* (21%) and *Staphylococcus aureus* (18%, of which 11% MRSA). We can notice the similarities with our study, when it comes to causes of neonatal infections. Especially interesting study of our region, recently was conducted in neighboring Serbia, in Kragujevac [17] where the most common diagnosis...
of neonatal patients in this study were: Pneumonia (64.9%), Infectio tractus urinaria (20.3%), sepsis (9.5%) and Omphalitis (5.4%). Compared with our study, where we also had neonates diagnosed with Pneumonia and Sepsis, the percentage distribution of the above diagnosis is different. The difference between these two studies is noticeable and the percentage representation of causes of neonatal infections. In a study from Serbia, the most common causes were: Klebsiella-Enterobacter (39.3% of the total number of agents), Escherichia coli (25%) and CoNS (16.1%). Although the most common causes in Serbia were also the cause of infection in our study, their percentage representation is very different. A study conducted at the University Clinic in Sao Paulo [18], which had dealt with the incidence of MRSA in the intensive neonatal care, showed results similar to those in our study when it comes to time reporting symptoms of infection in neonates in relation to their age. This study is also equipped with several limitations. In essence, we do not know what the potential impact on the occurrence of infections. Since there is no information what they were exposed neonates before the onset of symptoms, we cannot determine the cause of the increase in the number of agents in certain periods. We do not know whether in the course of the hospital stay was on parenteral nutrition, have some of the catheter, underwent an invasive diagnostic procedures, mechanical ventilation or intubation. We do not know the real cause of disease in order to be able to connect the cases and determine whether there was an epidemic in the period increase in the number of cases of neonatal infection, and how to prevent. Also, we do not have data about the comorbidity of neonates to be able to establish a causal relationship between infection and their outcomes.

**Conclusion**

The neonates, who are hospitalized in the Neonatal Intensive Care Division, are population with the highest risk of neonatal infections which are significant cause of their morbidity and mortality. For that reason, it should be taken care to prevent the development infections in neonates.

**Declaration of interest**

The authors declare no conflicts of interest.

**References**


