REZUMAT

Introducere: Sepsisul este o cauză importantă de mortalitate și morbiditate la nou-născuți, în special la prematuri. Deși există diferite antibiotice folosite în tratamentul sepsisului neonatal, au apărut și specii rezistente. Obiective: Obiectivele studiului au fost identificarea bacteriilor responsabile de apariția sepsisului neonatal (SN) și determinarea sensibilității la antibiotice. Factorii de risc asociati cu SN au fost analizați, de asemenea. Material și metode: Au fost studiați 1910 nou-născuți internați în Sectiile de Neonatologie din Spitalul de Copii „Louis Ţurcanu” Timișoara, în perioada ianuarie 2008-iunie 2009. Au fost analizate foile de observație și antibiogramele nou-născuților. Rezultate: Cincizeci și nouă de nou-născuți au fost diagnosticăți cu SN. Dintre aceștia, 89,83% au fost prematuri, 52,54% au avut greutatea la naștere <2500g, iar 20,33% au fost mici pentru vârsta gestațională. Săizeci și una de bacterii au fost identificate din hemoculturi, dintre care cele mai frecvente au fost Serratia marcescens (49,18%), Klebsiella pneumoniae (14,75%), Pseudomonas aeruginosa (13,11%) și Staphylococcus aureus (8,19%). Bacteriile Gram-pozitive au fost 100% sensibile la Vancomycin, 75% la Linezolid și 66% la Clindamycin, iar bacteriile Gram-negative au fost sensibile la Imipenem (91,83%), Ciprofloxacin (90%) și au prezentat un nivel crescut de rezistență la Cefalosporine (89,37%-93,55%). Dintre factorii asociați cu SN putem enumera manevrele invazive la care au fost supuși nou-născuții, prematuritatea, greutatea la naștere<1500g sau ruperea prematură de membrane amniotice. Concluzie: Sepsisul este o problemă majoră pentru nou-născuți și prematuri. Este necesară elaborarea și aplicarea unor programe de monitorizare a rezistenței la antibiotice, pentru a evita răspândirea acesteia. Cuvinte cheie: sepsis neonatal, prematuri, sensibilitate la antibiotice

ABSTRACT

Introduction: Sepsis is a significant cause of mortality and morbidity in neonates, especially in preterm babies. Although there are various antibiotics to treat neonatal sepsis (NS), resistant strains have subsequently emerged. Objectives: The main objectives were to identify the culprit bacteria responsible for NS and to determine their antimicrobial sensitivity. We also analyzed risk factors associated with NS. Material and Methods: From January 2008 to June 2009, 1910 babies (age < 30 days of life) admitted to our Neonatal Departments of “Louis Turcanu” Children Emergency Hospital, Timisoara comprised the study lot. Medical and microbiology data were reviewed. Results: Fifty-nine newborns had NS. Their mean age was 5.7 days. Out of these, 89.83% were prematures, 52.54% had body weight < 2500g and 20.33% were small for gestational age. They presented several risk factors for developing NS. Sixty-one microorganisms were isolated from positive blood cultures, with a predominance of Serratia marcescens (49.18%), Klebsiella pneumoniae (14.75%), Pseudomonas aeruginosa (13.11%) and Staphylococcus aureus (8.19%). While Gram-positive bacteria were 100% sensitive to Vancomycin, 75% to Linezolid and 66% to Clindamycin, Gram-negative bacteria were sensitive to Imipenem (91.83%), Ciprofloxacin (90%) and showed high resistance levels to Cephalosporins (89.37%-93.55%). Dintre factorii asociati cu SN putem enumera manevrele invazive la care au fost supuși nou-născuții, prematuritatea, greutatea la naștere<1500g sau ruperea prematură de membrane amniotice. Conclusion: Sepsis is a major concern in neonates and preterm babies in our hospital. None of the antibiotics had 100% efficacy against Gram-negative bacteria. Hence, great care and implementation of special control programs is required to avoid further spread of antibiotic resistance. Key Words: neonatal sepsis, preterm baby, antibiotic sensitivity

INTRODUCTION

Sepsis prevails to be a major threat for neonates. Neonatal sepsis (NS) is an important cause of mortality and morbidity in neonatal intensive care units. The incidence of NS is approximately 1-5 per 1,000 newborns, with a wide range in preterm babies with birth weight under 1500 g and a mortality rate of 5–20%. Incidence of NS varies in regard with the characteristics of the hospital and of the newborns.
treated, as well as the type and duration of antibiotics and invasive procedures used for treatment. Although high, the mortality rate associated with NS is extremely variable, ranging from 12% to 81%, depending on the population and etiologic agent studied. The susceptibility of NS is multifactorial and complex. Risk factors include interaction of materno-fetal colonization, transplacental immunity, physical and cellular defense mechanisms of the neonate (immaturity in humoral, phagocytic and cellular immunity), hypoxia, acidosis and metabolic disorders.

Classification of sepsis in neonates and preterm babies is in two categories, depending on the onset of infection: early onset sepsis (EOS) having occurrence in the 1st week of life, and late onset sepsis (LOS) with an onset after the first week of life. EOS is mainly acquired before delivery (vertical transmission from organisms that infect/colonize the maternal genital tract or via the placenta from maternal bacteremia) or during delivery as the baby passes through the birth canal. Whereas LOS found in newborn is either due to poor environmental conditions or medical and surgical treatments instituted to the child in order to save his life. The neonatologist should keep in mind that, no doubt, medical science is advancing, but its side effects are running in parallel too. Hence, before providing life supportive maneuvers to the sick newborn, precautions and ways to fight with side effects should be mandatory as well and preplanned in accordance to their weak immune system.

Because early symptoms in neonates are not specific, identification of NS is quite challenging for the neonatologist. Since delayed treatment raises the risk of mortality, whenever maternal risk factors for NS are suspected or the newborn manifests symptoms suggestive of an infection, cultures should be obtained and antibiotic therapy should be initiated immediately, which can prove to be beneficial on a large extent. Inappropriate uses of antibiotics leads to the development of multiresistant bacteria strains in the hospital setting. Active surveillance of infected newborns and the antimicrobial sensitivity testing of the responsible pathogenic microorganisms is important in defining the empiric antibiotic regimens to be followed in future practice.

OBJECTIVES

The objective of our study was to find out the common pathogens responsible for NS in our hospital and to determine the antimicrobial sensitivity patterns of these culprits. In addition, we studied the risk factors associated with NS.

MATERIAL AND METHODS

We conducted our study in the Neonatal Departments of “Louis Turcanu” Children Emergency Hospital, Timisoara, from January 2008 to June 2009. Data from neonates and premature babies (<30 days of life) admitted and having performed blood cultures were analyzed. Our study complies with the Declaration of Helsinki and has been approved by our institutional Ethics Committee.

The following were inclusion criteria:

- Clinical signs and symptoms of sepsis
  - hypoactivity, lethargy
  - hypothermia or hyperthermia,
  - pallor, cyanosis, jaundice, cutis marmoratus,
  - immature/total neutrophils ratio >0.2,
  - hepatosplenomegaly,
  - poor peripheral circulation,
  - apnea,
  - hepatosplenomegaly,
  - vomiting, abdominal distention and diarrhea,
  - poor sucking

- Laboratory findings
  - leucopenia (<5000/mm³) or leucocytosis (>25000/mm³),
  - neutropenia (<1750/mm³),
  - thrombocytopenia (<15000/mm³),
  - immature/total neutrophils ratio >0.2,
  - hypo- or hyperglycemia,
  - positive blood cultures,
  - positive inflammatory tests.

Collecting blood cultures on suspicion of NS and performing antibiotic susceptibility on those found to be positive for a specific organism were our main goals. Blood specimens were cultured on Mac Conkey agar and incubated for 24 hours at 37°C. Bacterial pathogens were identified using standard biochemical methods. The antimicrobial sensitivity of the isolates was determined by the disk diffusion method, using the following disks: Ampicillin, Penicillin, Oxacillin, Erythromycin, Clindamycin, Trimethoprim/Sulfamethoxazole, Amoxicillin / Clavulanate, Ticarcillin /Clavulanate, Gentamicin, Amikacin, Cefotetan, Cefazolin, Cefotaxime, Cefuroxime, Cefazidime, Ceftriaxone, Ciprofloxacin, Moxifloxacin, Levofloxacin, Vancomycin, Linezolid, Imipenem, Meropenem, Ertapenem and Colistin. Quality control tests used standard strains of Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27953 and Staphylococcus aureus ATCC 25923. Identification of multidrug bacteria such as MRSA isolates and ESBL-producing strains was according to NCCLS standards.

Furthermore, the following information were collected from patients found to be with positive blood
cultures: gender, gestational age, birth weight, APGAR score, clinical diagnosis, length of hospitalization, pathogenic bacteria isolated from positive cultures and their antibiotic susceptibility. In addition, we recorded information regarding the maternal health status (premature or prolonged rupture of membranes, infections during pregnancy or labor). Evaluations such as echocardiography or CT scan were done if needed.

We considered a newborn as preterm baby if its gestational age was less than 36 weeks. Small for gestational age referred to a newborn with gestational age over 36 weeks but its birth weight less than 2500g. Incidence of NS represents all the newborns with positive blood cultures. The overall mortality rate represented the total number of newborns death among the total number of patients, while the case fatality rate defined the number of deaths among newborns with NS.

RESULTS

Patient population

A total of 1,910 newborns were hospitalized during the study period. Out of these, 59 patients had clinical signs and laboratory data characteristic of NS. Table 1 shows the general demographic data of the hospitalized newborns presenting NS.

Table 1. Demographic data relating to the hospitalized newborns with NS

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (± SD) days</td>
<td>5.7± 4.3</td>
</tr>
<tr>
<td>Male gender</td>
<td>31 (52.47%)</td>
</tr>
<tr>
<td>Twins</td>
<td>7 (11.86%)</td>
</tr>
<tr>
<td>Gestational age (± SD) weeks*</td>
<td>33,68±3.30 (26-38)</td>
</tr>
<tr>
<td>Birth weight (± SD) grams*</td>
<td>1839±626.06 (800-3530)</td>
</tr>
<tr>
<td>Normal birth weight (BW &gt; 2,501 g)</td>
<td>47.45%</td>
</tr>
<tr>
<td>Low birth weight (BW 1,501-2,500 g)</td>
<td>37.28%</td>
</tr>
<tr>
<td>Very low birth weight (BW 1,001-1,500 g)</td>
<td>13.55%</td>
</tr>
<tr>
<td>Extremely low birth weight (BW &lt; 1000 g)</td>
<td>1.09%</td>
</tr>
<tr>
<td>Small for gestation age</td>
<td>12 (20.33%)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>45 (76.27%)</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>14 (23.72%)</td>
</tr>
<tr>
<td>APGAR score*</td>
<td>14 (23.72%)</td>
</tr>
<tr>
<td>1 min</td>
<td>6 (2-8)</td>
</tr>
<tr>
<td>5 min</td>
<td>8 (5-10)</td>
</tr>
<tr>
<td>Length of stay (days)*</td>
<td>29.5 (4-57)</td>
</tr>
<tr>
<td>Death</td>
<td>6 (10.16%)</td>
</tr>
</tbody>
</table>

*median (range)

The mean age of newborns studied was 5.70 days, while the mean gestational age was 33.68 weeks. Among these, 89.83% were preterm babies and seven neonates were from five pairs of twins. Male newborns were more prevalent than females. Birth weights (BW) ranged between 800-3530 grams, while small for gestational age babies represented 20.33% of them. Vaginal deliveries were frequently associated with NS compared with C-Section deliveries. The median of hospital stay was 29.5 days. All neonates had survival and adaptation problems in the first minutes of life.

The most important comorbidities associated to prematurity comprised congenital heart malformations, neurological disorders, respiratory distress, or renal and hepatic failures, as shown in Table 2.

Table 2. Underlying diseases which lead to or accompany NS

<table>
<thead>
<tr>
<th>Underlying risk conditions</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prematurity</td>
<td>53 (89.83%)</td>
</tr>
<tr>
<td>Birth weight (BW&lt;2500)</td>
<td>31 (52.54%)</td>
</tr>
<tr>
<td>Very low birth weight (VLBW; BW&lt; 1500g)</td>
<td>31 (52.54%)</td>
</tr>
<tr>
<td>Prolonged amniotic membrane rupture time&gt;8h</td>
<td>13 (21.31%)</td>
</tr>
<tr>
<td>Congenital heart diseases</td>
<td>16 (27.11%)</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>14 (23.72%)</td>
</tr>
<tr>
<td>Hepatic and renal failure</td>
<td>7 (11.86%)</td>
</tr>
<tr>
<td>Neurological malformations</td>
<td>5 (8.47%)</td>
</tr>
<tr>
<td>Seizures</td>
<td>4 (6.77%)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>2 (3.38%)</td>
</tr>
<tr>
<td>Orotracheal intubation and mechanical ventilation</td>
<td>9 (15.25%)</td>
</tr>
</tbody>
</table>

Also all neonates underwent different invasive procedures in order to save their life such as vascular catheters for the administration of antibiotics, blood or parenteral nutrition, nasogastric tubes for feeding or urinary catheters in renal failure; 15.25% of neonates had an endotracheal tube and were mechanically ventilated. Unfortunately, these maneuvers provide a pathway for microorganisms to enter the body.

The overall incidence of NS in our hospital was 3.08% (59/1,910). The overall mortality rate was 0.31% (6/1,910), while the case fatality rate was 10.16% (6/59). Among the cases that died, one child had BW less than 1000 g and gestational age of 26 weeks. The rest had BW under 1500g and all were preterm. They had NS with S. aureus, Klebsiella pneumoniae, or Pseudomonas aeruginosa. One had a polymicrobial sepsis with Klebsiella and Pseudomonas. In these
cases, underlying diseases accompanying NS were complicated congenital heart disease, meningitis and seizures, perinatal asphyxia or respiratory distress.

**Distribution of sepsis**

Blood cultures revealed sixty-one positive strains. A mean of 1.01 strains were isolated per patient. A single neonate had a polymicrobial sepsis with Klebsiella pneumoniae and Pseudomonas aeruginosa and another had two episodes of sepsis, one with Enterococcus faecium and the other with S. epidermidis.

Gram-negative bacteria accounted for 81.96% of isolated strains. Serratia marcescens (49.18%), Klebsiella pneumoniae (14.75%) and Pseudomonas aeruginosa (13.11%) were the most frequently encountered species. Other Gram-negative isolates (4.92%) were E. coli, Klyuyvera ascorbata and Enterobacter. Among Gram-positive strains (18.03%), we found a predominance of Staphylococcus aureus (8.19%), followed by Enterococcus faecium and Coagulase negative Staphylococci (4.92% each one). Candida albicans infections were associated with bacteria in 10.16% of cases. We encountered no isolate of group B streptococci (GBS). EOS represented only 21.31% of total NS and LOS were the rest. S. aureus, Coagulase negative Staphylococci and Enterococcus faecium were the majority (69.23%) in the EOS, while Gram-negative were dominant in the LOS (93.75%). Enterococcus faecium, S. aureus, Coagulase negative Staphylococci, Klebsiella pneumoniae and Pseudomonas aeruginosa affected both groups. E. coli was characteristic for EOS, while Klyuyvera ascorbata, Enterobacter and Serratia marcescens were found only in LOS. The incubation time for clinically significant bacterial blood cultures ranged from 24 h to 144 h and the median time of positivation of blood culture was 68 hours (table 3) We have found no correlation between the time of positivation and bacteria virulence (p=0.01).

**Table 3. Time of positivation of blood cultures**

<table>
<thead>
<tr>
<th>Time of positivation of blood cultures</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>48h</td>
<td>23 (37.70%)</td>
</tr>
<tr>
<td>72h</td>
<td>27 (44.26%)</td>
</tr>
<tr>
<td>&gt;72h</td>
<td>11 (18.03%)</td>
</tr>
</tbody>
</table>

**Antibiotic susceptibility**

Gram-negative bacteria were sensible to Carpenems and Fluoroquinolones as shown in Figure 1 and were highly resistant to many antimicrobial drugs particularly to the Cephalosporins (Ceftazidime 89.37% and Ceftriaxone 93.55%) and Trimethoprim/Sulfamethoxazole (89.19%). Intermediate resistance was with Colistin (41.18%) and Ticarcillin/Clavulanate (58.34%).

**Figure 1. Antibiotic susceptibility of Gram-negative organisms**

Serratia marcescens, the leading bacteria isolated, was susceptible to Ciprofloxacin, Moxifloxacin, Imipenem and Meropenem (91.66%, 91.30%, 90% and 86.66%, respectively). Cefotetan was the only drug from the Cephalosporins group these bacteria were sensible to (78.57%). Resistance rates over 90% were found in the 3rd and 4th generations Cephalosporins and Aminoglycosides. Klebsiella pneumoniae strains were 100% sensitive to Colistin, 88.88% to Meronem and Imipenem and 87.50% to Ciprofloxacin. Intermediate susceptibility was found to Aminoglycosides and beta-lactam/beta-lactamase inhibitor combinations (Ticarcillin/Clavulanate). Out of them, one-third were extended spectrum beta-lactamase (ESBL)-producing strains. All Pseudomonas aeruginosa isolates were sensible to Imipenem, while 85% of them were susceptible to Gentamicin and Ciprofloxacin. Enterobacter, E. coli and Klyuyvera ascorbata strains were less resistant to Carbapenens and Ciprofloxacin.

Gram-positive strains were 100% sensible to Vancomycin. Sensibility to Linezolid, Clindamicyn and Levofloxacin was as presented in Figure 2. S. aureus and Coagulase negative Staphylococci had 50% resistance to Oxacillin, while to Erythromycin they were sensible in 33.33% of cases. All strains presented high resistance rate to Ampicillin and Penicillin. Out of S. aureus isolates, we found a single strain of Methicillin-resistant S. aureus (MRSA) and a single Methicillin-sensible S. aureus (MRSSA). No Vancomycin-resistant Enterococcus isolates were encountered.

**Figure 2. Antibiotic sensibility of Gram-positive bacteria**
DISCUSSION

Newborns examined in this study presented several risk factors for contracting NS. The most important predisposing factors for NS were:

1. Appliance of invasive procedures, such as tracheal intubations, mechanical ventilation, airway aspiration or indwelling vascular catheters, which alter skin or mucous membrane barriers, and

2. Presence of conditions such as prematurity, BW<1500g, small for gestational age, prolonged amniotic membrane rupture, congenital heart malformations, neurological disorders or septic delivery.

Other studies showed that prematurity and VLBW significantly influenced the incidence of NS while a significant decrease in the risk of septicemia was associated with the increase in BW and gestational age. Male gender presented an increased rate of association with NS. Maternal genital tract infection is an important cause of preterm labor, with an increased risk of vertical transmission in newborn. Administration of intrapartum antibiotic prophylaxis during deliveries explains the reduction of EOS found in our study.

The microorganisms frequently encountered in our study were similar with those reported in previous studies. Medical literature presented that KES group (Klebsiella spp., Enterobacter spp. and Serratia spp.) comprised pathogens of significant morbidity and mortality in immunocompromised and debilitated patients, as well as newborns, and these bacterial species frequently transfer their resistance genes to susceptible strains during outbreaks.

Generally, Serratia marcescens does not constitute part of the intestinal bacterial flora of neonates. Medical literature presented different ways of transmission to neonates, such as feeding, use of soaps, breast pumps, as well as through direct patient contact. It can cause rapidly spreading outbreaks of severe and potentially fatal infections in neonatal units, particularly as bloodstream infections. Serratia species constitutively possess chromosomal inducible AmpC beta-lactamase and have the ability to develop resistance rapidly to many beta-lactam antibiotics. After the introduction of 3rd generation Cephalosporins in medical practice, multidrug resistant strains of Serratia emerged as a cause of bacteremia. Risk factors for acquisition are low birth weight, long duration of hospitalization and critical care assistance.

The results of this study underlined the high rate of resistance to 3rd and 4th generation Cephalosporins (89.37%-93.55%) and an intermediate resistance level to Aminoglycosides among Gram-negative strains isolates in our hospital. This fact suggests the existence of a variety of resistance mechanisms, such as reduced permeability of antimicrobials through the outer membrane, enzyme production and efflux pump mechanisms, as described by other authors. The high resistance levels found could be explained by the high frequency of Cephalosporins use for both prophylactic and therapeutic reason in hospitalized newborns.

Our study revealed that one-third of Klebsiella pneumoniae isolates were ESBL-producing strains. ESBL phenotypes are complex, due to the production of multiple enzymes including inhibitor-resistant TEM enzymes, AmpC beta-lactamase, enzyme hyperproduction and porin loss.

A number of screening procedures, such as C reactive protein, procalcitonin, IgM, thrombocytopenia, absolute neutrophils count and the relation of immune to total cells, are helpful for diagnosis of NS. Cytokines and surface markers (IL-6, IL-8, and IL-10) are accurate for establishing this diagnosis, but the costs of tests are barriers of its day-to-day use. However, blood culture remains the “gold standard” for the definite diagnosis of sepsis, despite recent advances in the molecular diagnosis of infection.

The important role played by human milk in the immune system of preterm babies consists in fewer infections and shorter hospital stay. Oral lactoferrin prophylaxis (a normal component of human colostrum) reduces the incidence of LOS in infants weighing less than 1500 g and is most effective in infants weighing less than 1000 g.

Various bacterial and fungal agents colonize hospitalized babies, healthcare workers, and visitors. Transmission of pathogenic agents is either by direct contact or indirectly via contaminated equipments, intravenous fluids, medications, blood products or enteral feedings. Colonization of the infant’s skin, umbilicus, respiratory tract, or gastrointestinal tract with pathogenic agents often precedes the development of infection. Antibiotic use interferes with colonization by normal flora, thereby facilitating colonization with more virulent pathogens.

The pattern of bacterial organisms constantly changes with time and place. Previously sensitive organisms are rapidly becoming resistant to commonly used antibiotics due to inappropriate use, thus making the treatment difficult and expensive. At present, in the developing countries, Gram-negative organisms remain to be the major threat. These culprits have...
developed multidrug resistance over the last two decades.\textsuperscript{29} The main reasons for this resistance are indiscriminate and irrational use of antibiotics, over the counter sale of antibiotics and poor infection control procedures in maternities.

CONCLUSIONS

Life expectancy of neonates is increasing with the help of advances in neonatology, new life support techniques and new treatment modalities. Because of increased life expectancy, duration of hospitalization is also increasing and in spite of new antibiotics and new supportive measures, LOS and its treatment has become a major problem in neonatal departments. To overcome this problem, it is important to know the distribution of etiological agents and their antimicrobial sensitivity. Continued surveillance is mandatory to detect these temporal changes in spectrum and sensitivity of causative organisms, which will help in treating the NS and eventually will reduce the neonatal morbidity and mortality. A revised infection control program with emphasis on hand washing techniques and antibiotic cycling helps to control these infections.

REFERENCES