Clinical and microbiological characteristics of pediatric Gram-negative bloodstream infections

Doctoral thesis

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INTRODUCTION

Infectious diseases have been major cause of morbidity and mortality since the earliest times in the history of mankind. Fight against micro-organisms remains in the focus of modern medicine despite the success of vaccination and development of antibiotics in the previous century. Although improvement in social and hygienic circumstances of the population caused a marked reduction in the frequency of life threatening infections, appearance of new challenges such as antimicrobial resistance and vulnerable patient groups (e.g. immunocompromised hosts, and critical care patients with multiple diseases and invasive devices) suggest that vigilance regarding the combat against infection cannot be less intense nowadays.

The spectrum of bloodstream infections (BSIs) ranging from mild infection to severe sepsis and septic shock causes high mortality, longer hospitalizations, and excessive cost to the patients and healthcare system. Gram-negative bacteria are responsible for a great portion of bloodstream infection, and an increasing incidence of sepsis caused multidrug-resistant (MDR) Gram-negative strains has been detected in the last decades.

Studies demonstrated that Gram-negative pathogens account for 30-55% of all bloodstream infections detected in children depending on different age-groups, patient population and geographical region. Data about etiology, clinical characteristics and outcome of pediatric Gram-negative BSIs in Hungary are scarce, as are in other Central European countries. However, it is known that hospital acquired infections caused by antibiotic-resistant Gram-negative bacteria have been increasing in Hungary in the last decades, and Gram-negative infections and antimicrobial resistance is a major concern in this region as well. To evaluate pathogenic agents, antimicrobial resistance, clinical severity, and outcome of Gram-negative BSIs in Hungarian children we conducted a two-year multicenter study in several pediatric healthcare institutions in Budapest, Hungary. The main aim of the study was to identify risk factors of acquisition of BSIs caused by multidrug-resistant Gram-negative bacteria, and determine factors associated with poor outcome that can influence the therapeutic approach to these children in the future.

AIMS

1. **Data collections focusing on microbiology of pediatric Gram-negative BSIs**
    - Analysis of pathogen distribution in community acquired (CA) and nosocomial/healthcare associated (HCA) pediatric BSIs in our population
    - Comparison of CA and nosocomial/HCA pediatric BSIs in different patient groups and setting
    - Analysis of antimicrobial resistance patterns of pathogens causing pediatric BSIs
    - Determination of factors associated with antimicrobial resistance and multidrug-resistance in pediatric BSIs
    - Identification of vulnerable patient groups among children that are prone to acquire BSIs caused by multidrug-resistant pathogens

2. **Clinical characterization of pediatric Gram-negative BSIs**
    - Description of clinical course and severity of pediatric Gram-negative BSIs
    - Determination of frequency of organ failures related to pediatric Gram-negative BSI in different patient groups

3. **Determination of factors influencing outcome of pediatric Gram-negative BSIs**
    - Epidemiological factors (e.g. age, underlying disease, department of care, previous medication, medical care and interventions)
    - Effects of microbiological factors on outcome (pathogens, multidrug-resistance)
    - Effects of appropriate or inappropriate empiric antimicrobial treatment on the outcome of pediatric Gram-negative infections

4. Analysis of outbreaks caused by Gram-negative bacteria in children

- Identification of pathogens that cause outbreaks in children during the study period
- Analysis of the spread and determination of the source of the epidemic strain
- Description of special clinical characteristics in infections related to the outbreak

METHODS

Patients, setting, and factors investigated

We conducted a two year observational, multicenter in the following pediatric institutions in Budapest, Hungary:

- Semmelweis University of Budapest, 1st Department of Pediatrics (Intensive Care Unit, Perinatal Intensive Care Unit, Neonatal Surgery, Infant Care Unit)
- Semmelweis University of Budapest, 2nd Department of Pediatrics (Dept. for Hematology, Dept. for Oncology, Dept. for Neuro-oncology, Dept. for Surgery, Intensive Care Unit, Microbiology Laboratory)
- Egyesített Szent Iván and Szent László Hospital (Pediatric Intensive Care Unit, Dept. for Pediatric Infectious Diseases, Dept. for Pediatric Hematology and Stem Cell Transplantation, Bacteriology Laboratory)
- Gottsegen György Hungarian Institute of Cardiology, Center for Pediatric Cardiology (Pediatric Cardiology Intensive Care Unit, Pediatric Cardiac Surgery Intensive Care Unit)
- Heim Pál Childrens Hospital, Madararász utcai Dept. (Intensive Care Unit)
- MRE Bethesda Childrens Hospital (Dept. for Anaesthesiology and Intensive Care)
- Semmelweis University, Institute of Medical Microbiology

During the study period from 1st December 2010 to 31st December 2011 we collected data on patients <18 years of age with Gram-negative bloodstream infection at the study sites with active surveillance. Patients with a BSI caused by Enterobacteriaceae

or non-fermentative Gram-negative bacteria were enrolled in the study. We excluded blood cultures positive for *Haemophilus spp, Neisseria spp.* and *Salmonella spp.* as we focused on pathogens that may cause both community-acquired and nosocomial infections. Cases were excluded from the clinical data analysis also (1) if the same organism was cultured from the blood of the same patients within 4 weeks of the first episode of BSI and (2) if parents refused consent for study participation.

Patients enrolled at the different study sites were pooled and categorized into four groups according to type of department as follows: 'NICU', 'PICU' (patients from the neonatal and pediatric ICUs, pediatric cardiac and pediatric cardiac-surgery ICU), 'Hematology-oncology' (patients from all hematology oncology and stem cell transplantation units), and 'Other' (patients from the General Pediatric Ward, Surgery and Neonatal Surgery Ward).

The following data have been collected from the medical charts of the enrolled patients: age, gender, gestational age in cases of infants <3 months of age, co-morbidities, malignancy (solid tumor or hematological malignancy), antineoplastic therapy, solid or stem cell transplantation, time from the hospital admission to the collection of blood for culture, previous hospitalization in the last 60 days, antimicrobial treatment in the last 30 days, antimicrobial treatment at time of the blood culture test, initial antimicrobial therapy after the collection of the blood for culture, change in antimicrobial therapy after the results of the blood culture, focus of the infection; clinical severity (BSI/sepsis, severe sepsis, septic shock), organ dysfunction; mechanical ventilation, peripheral or central venous access, intra-arterial line, urinary catheter, surgical lines, parenteral nutrition or blood transfusion prior to the time of blood culture; case fatality rate (CFR), hospital discharge or continued hospital treatment was determined at the 28th day after the detection of BSI. BSI-related fatality was also determined, it was defined as death caused by deterioration of patients' clinical condition due to the BSI observed in the study, and other causes of death were excluded after reviewing the medical records of patients.

Microbiology methods and antimicrobial susceptibility testing

Blood culture specimens were inoculated into BacT/ALERT PF Pediatric FAN or BacT/ALERT FA FAN culture bottles using the BacT/ALERT 3D automated

microbial detection system (bioMerieux, France). Clinical isolates were identified by API 20E, VITEK 2 System and ID strips using the on line bacteria database (BioMérieux, France).

Susceptibility to antimicrobial agents was determined by standard disc diffusion method (Mast Diagnostica, Germany) and Etest (bioMérieux France). Detection of extended-spectrum β-lactamase and AmpC productions were performed with ESBL&AmpC Detection Disc Sets (Mast Diagnostica, Germany) and with ESBL Etest (bioMerieux, France), according to the manufacturer's instructions.

Antimicrobial susceptibility tests were confirmed at Institute of Medical Microbiology, Semmelweis University by determination of minimal inhibitory concentrations (MICs) by microdilution method for the following antibiotics: amoxicillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefotaxim, ceftriaxone, cefepime, imipenem, meropenem, gentamicin, tobramycin, trimethoprim/sulfomethoxazol and ciprofloxacin. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 was used as the reference strain for antimicrobial susceptibility testing in case of Enterobacteriaceae. Antibiotic susceptibility results were performed and interpreted according to the guideline of European Committee on Antimicrobial Susceptibility Testing (EUCAST).

The clonal relationships of the *S. marcescens* isolates were studied by pulsed-field gel electrophoresis (PFGE) at the Department of Phage and Molecular typing, National Center for Epidemiology. The PFGE method was performed in line with the standardized CDC protocol using CHEF DR II (Bio-Rad, USA), and using restriction endonuclease XbaI. Gels were interpreted with Fingerprinting II Informatix Software (Bio-Rad, USA). Levels of similarity were calculated with the Dice coefficient, and UPGMA ('unweighted pair group method with arithmetic averages') was used for the cluster analysis of the PFGE patterns. Pulsotypes (PTs) were defined at 85% similarity between macrorestriction patterns and marked by letters and numbers. Clonally related isolates were supposed if they belonged to the same PT.

Statistical analysis

Categorical data are presented as numbers and percentages, continuous data as median and interquartile range. For the univariate analysis of variables Pearson's Chi-square

tests and Fisher's exact tests were used. For comparison of continuous variables Mann-Whitney U test was used. Factors associated with multidrug-resistance and mortality were also evaluated using a stepwise multivariate logistic regression analysis. Results were considered significant if $p<0.05$. Statistical analysis was performed by using SPSS Statistics 22 (IBM Inc., Chicago, IL, USA) software.

Ethical issues

This study has been approved by the *Scientific and Research Ethics Council* of the Hungarian Medical Research Council under the reference number 845-0/2010-1018EKU (39/PI/010.). Informed consent has been attained from the parents of all infants and children whose clinical data has been used in this study.

RESULTS

Demographical data and underlying conditions

A total of 134 GN-BSIs fulfilled the inclusion criteria, and were enrolled in the data analysis. Only 7 cases (5.2%) of the BSIs were community acquired (CA), hence principally hospital acquired (HA) and healthcare associated (HCA) infections were detected during the study. Boys represented 61.9 % of the children**;** median age was 0.5 year (range 1 day to 17.3 years, IQR 0.6-6.17). Proportion of infants <3 month of age was 44%. Most patients were recruited at PICUs (33.6%), and hematology-oncology wards (27.6%), NICU patients reached 23.1% of all children.

Most children (124 of 134 cases; 92.5%) had at least one underlying illness, that included e.g. hematologic malignancies (n=23), solid tumors (n=21), primary immunodeficiencies (n=3), congenital anomalies (cardiac, GIT), chromosomal defects, chronic respiratory disease, trauma, and burns. Out of 29 neonates 18 (62.1%) were premature at birth.

Only 1 of the 7 cases with community acquired BSIs had a predisposing condition, whereas 96.9% of children with HA/HCA infection had an underlying disease or co-morbidity. Forty-four children (32.8% of all cases) had malignancy, and 12 (27.3%) of them had a relapse of their primary disease at the time of the BSI. Twenty-nine

percent of the patients received antineoplastic treatment before the acquisition of BSI. Chemotherapy induced neutropenia could be detected in 28.4% of cases; 9.7% of cases had persistent neutropenia (>10 days).

Severity and organ failures

In 47% of patients no organ failure could be detected, severe sepsis and septic shock developed in 26.1% and 26.9 %, respectively. Severe cases were more frequent in the age group 2-5 years and in children >5 years of age. At least one organ failure developed in 54.1%, respiratory and circulatory system was most frequently affected. In children <3 months of age rate of respiratory and circulatory insufficiency were higher than in older children, but renal and liver failure was more frequent in the latter age group. Factors associated with the development of septic shock were urinary catheterization, transfusion, and polymicrobial infection in univariate analysis, and mechanical ventilation (OR 2.68, CI 1.20-6.46; p=0.048) and multidrug-resistant infection (OR 3.06, 1.34-7.01; p<0.01) in multivariate analysis.

Focus of infections, prior interventions

Source of BSI could be identified in 38.8% of cases; cases were most frequently associated with central venous line infection (15%), intra-abdominal infection and urinary tract infections. In 14 patients BSI caused by the same *Serratia marcescens* strain developed as part of an outbreak without an identifiable source.

Most frequent medical interventions prior to BSIs were central venous catheterization (61.9%) parenteral nutrition (34.3%), transfusion (44%), and mechanical ventilation (30.6%). Twenty-nine percent of patients received anticancer chemotherapy, and 28% had chemotherapy induced neutropenia. Patients with neutropenia lasting >10 days represented 9.7% of all children. Patients were hospitalized in 85.8% in 60 days-period prior to the BSI.

Pathogens and antimicrobial resistance

We collected 145 Gram-negative blood isolates from 124 monomicrobial and 10 polymicrobial infections. Most frequent pathogens were *E. coli* (22.8%), followed by *Klebsiella spp*. (20%), and *P. aeruginosa* (16%). *Enterobacteriaceae* accounted for

68.3% of all infections, *Enterobacter spp*. (12%) and *S. marcescens* (12%) were frequent as well. Among Gram-negative non-fermentative bacteria *Pseudomonas spp*., *S. maltophilia* (4%), and *Acinetobacter spp.* (6%) could be detected most frequently. Among *Pseudomonas spp*. *P. aeruginosa* was isolated in 24/26 cases, the two remainder strains belonged to the *P. putida* species. In neonatal ICU *Enterobacteriaceae* represented 88% of all isolates, *Klebsiella spp.* (39.4%), *S. marcescens* (21%), and *E. coli* (12.1%) being the most frequent isolates. Frequency of *E. coli* was very high in hematology-oncology patients (47%), as was *P. aeruginosa* (24%). This pathogen was also prevalent in ICU patients. Other non-fermenatitve bacteria were isolated mostly in ICUs and hematology-oncology wards as well.

In all pathogens highest resistance rates was detected against amoxicillin, amoxicillin/clavulanin acid and cefuroxim (76.3%, 59.8% and 43.3%, respectively). Resistance against third generation cephalosporins (cefotaxime) reached 19.3%. Meropenem resistance was 11%, ceftazidime resistance was 15.9%, and piperacillin/tazobactam resistance was 21.1%. Among *Enterobacteriaceae Enterobacter spp*. had the highest resistance against cefotaxime, ceftazidime, cefepime and piperacillin/tazobactam (52.9%, 47.1%, 29.4% and 35.3%, respectively), but no carbapenem resistant strain was detected among *Enterobacter spp*. In *Enterobacteriaceae* the only species that showed meropenem resistance was *Klebsiella spp*. (one case). All *Pseudomonas spp*. isolates showed susceptibility to amikacin, and 88.5% resistance rates to both ceftazidime and cefepim. Meropenem resistance of *P. aeruginosa* strains was 20.8%. In children aged <3 months resistance rates were markedly lower compared to older children. Strains resistant to broad-spectrum beta-lactam antibiotics were more frequent in ICUs and hematology-oncology/stem cell transplantation wards. We detected no remarkable difference in the resistance rates between ICU and hematology-oncology patients, except for ciprofloxacin and trimethoprim/sulfomethoxazol. These antibiotics were less effective in hematology/oncology patients. Extended spectrum beta-lactamase (ESBL) production could be detected in *Enterobacteriaceae* strains, in 9.1%. ESBL production was most prevalent in *Enterobacter spp*. (17.7 %), *Klebsiella spp*. (10.3%) and *E. coli* (9.1%). Frequency of AmpC was highest also in *Enterobacter spp*. (11.8%), and *E. coli* (3%). Antimicrobial combination of

piperacillin/tazobactam+amikacin, meropenem+amikacin and ceftazidime+amikacin would be the most effective in ICU patients, and ceftazidime+gentamicin or ceftazidim+amikacin in hematology-oncology patients. Efficacy of these combinations was mostly dependent on the efficacy of the aminoglikoside component, amikacin showed higher *in vitro* efficacy in all sub-populations. Rate of multiresistance was 31% (45 isolates), which could be detected *E. coli* (48%), *Pseudomonas spp*. (31%), and *Enterobacter spp*. (47%) isolates. Thirty-three percent of all septic episodes were caused by MDR strains, the rate was higher in boys than in girls (40% vs. 24%), male gender was an independent risk factor for acquisition of MDR strains in multivariate analysis (OR 3.13, CI 1.19-8.23; p=0.021). Prevalence of multiresistance was higher in older children, median age of children with an MDR pathogen was 4.33 years compared to 0.24 years in children with a susceptible pathogen (p<0.001). Rate of MDR was 13.8% and 16.7% in newborns and infants, and 54.3% in children older than 5 years (p<0.005).

Hematology-oncology patients has higher risk for infection by MDR Gram-negatives compared to NICU patients (54.1% vs. 12.9%; OR 7.94, CI 2.31-27.3, p<0.01), as were children with neutropenia (55%). Multiresistance in children with neutropenia lasting >10 days had an extremely high rate of MDR (89%, p<0.01). Presence of malignant disease, previous anticancer chemotherapy and stem cell transplantation was associated with multiresistance (rates were 50%, 51% and 67%, respectively; p<0.05). Multiresistance rate was doubled in polymicrobial infections (70% vs. 30.6%; p=0.02). Rate of MDR in *E. coli* was significantly higher than in *Klebsiella spp.* (51.7% vs. 14.8%), OR was 5.89 (CI 1.62-21.4; p<0.01). More severe cases were more prone to have an MDR pathogen, septic shock was associated with the presence of multiresistance (OR 3.06, CI 1.34-7.01; p<0.01). MDR rate was also higher in patients with 4 or 5 organ failures (63%; OR 5.72, CI 1.62-20.2; p<0.01) than is less severe cases. Patients previously treated with cephalosporines were at high risk of acquisition of MDR pathogens (OR 3.55, CI 1.11-11.4, p=0.033). Empiric antibiotic treatment was more effective in non-MDR infections than in MDR-infections, and empiric treatment should be changed more frequently in the MDR group.

Outcome

Twenty-eight-day mortality was 17.9% (24 cases), sepsis-related mortality was 9.7% (13 children), 35.8% of children could be discharged from hospital, and 46.3% needed further hospital treatment. The highest case fatality rate (CFR) was detected in ICUs (33.3%), and hematology-oncology wards (13.5%), while CFR was 9.7% in NICU, and 4.8% in other wards. Odds of mortality in ICUs compared to the NICU, hematology-oncology, and other wards was 4.67 (p=0.025), 3.20 (p=0.04), and 10.00 (p=0.03), respectively. Mortality was lower in children <3 months of age than in older children, and older age was an independent risk factor for mortality in multivariate analysis (OR 1.1; CI 1.00-1.24; p=0.04). Highest CFR was observed in patients with neutropenia lasting >10 days (46.2%), and in children with malignancy (33.3%).

Septic shock was the strongest predictor of mortality (OR 13.03; CI 13.0-40.1; p<0.001). None of the patients without organ failures died, but mortality of children with at least one organ failure was 32.9%. CFR in patients with 1 and 2 insufficient organs was 10% and 17%, respectively, and CFR reached 60% in patients with 3 or more organ failures. Factors significantly associated with mortality were transfusion, parenteral nutrition, urinary catheter, and stem cell transplantation. In multivariate analysis parenteral nutrition was proved as an independent risk factor for mortality (OR 7.91, CI 2.41-26.00, p<0.001). Among monomicrobial infections *Pseudomonas spp*., *S. marcescens*, and *Enterobacter spp*. BSIs caused the highest mortality (27.3%, 25%, and 15.4%). Sepsis-related death was most frequent in *S. marcescens* infections (18.5%). Polymicrobial infection has markedly higher CFR than monomicrobial infections (16.1% vs. 40%; OR 3.47, CI 0.90-13.4, p=0.07). Infections caused by MDR strains had higher mortality (24.4% vs. 14.6%), although results were not significant (p=0.16).

Outbreak of *Serratia marcescens* bloodstream infections

From May to June 2010 and from August to September 2011 we observed two consecutive periods of an outbreak of bloodstream infection caused by *S. marcescens* at 1st Dept. for Pediatrics, Semmelweis University, Budapest, Hungary. NICU, PICU, Neonatal Surgery and Infant Care Unit were involved in the outbreak. We investigated the *S. marcescens* strains by PFGE for molecular epidemic analysis. All cases caused

by the epidemic strain ware collected (these were considered to be epidemic infections), and all other strains were also collected to understand the dynamics of the outbreak. In all four pulsotypes had been identified, the epidemic strain was the SM009 pulsotype. Twelve BSI cases due to the epidemic strain were detected, and two other sporadic BSIs also were observed between the two outbreak periods, that were caused by other pulsotypes. We observed 13 colonizations by the epidemic strain, and for comparison we investigated two other strains that were isolated before the outbreak periods. These latter strains belonged to the SM009 pulsotype.

The first period of the outbreak was observed from early May 2010 to middle June 2010, nine BSIs and six colonizations were detected in NICU patients. Between the two outbreak periods (June 2010-Aug 2010) two sporadic BSI have been observed caused by other serotypes at Neonatal Surgery ward, but two colonizations by SM009 strains were also observed. This epidemic strain was also isolated from a water tap culture at the Infant Care Unit in November 2010. A neonate was carried the SM009 strain for a long period, the strain could be isolated from his samples at 41. and 164. days of the outbreak. During the second period of the in August 2011 (487. day from the first BSI) 3 BSIs and two colonizations were observed at the NICU and ICU wards. The first case during this period was admitted from another's' hospital neonatal ward, and the blood culture was positive for the epidemic S. marcescens strain in 24 hours from the admission. After the end of the second outbreak period the SM009 strain could be isolated from two asymptomatic carriers, but BSI did not develop until the end of study period. Epidemic BSIs were caused by the SM009 pulsotype during both the first and second outbreak period. Between the two outbreak periods two sporadic BSI were detected, the causative strain were related to other pulsotype, but carriage of SM009, could be demonstrated in this period as well, and an environmental culture also yielded this strain. In conclusion, the epidemic strain could be detected in the department before, during, between, and after the two outbreak periods. To control the outbreak, infection control team including neonatologist, infectologist, epidemiologist and microbiologist started to work at the department. Hand hygiene education was re-introduced, and the compliance of the healthcare workers was measured by regular audits. Barrier precautions and patient isolation was strengthened. Environmental cultures were taken, including healthcare workers'

hands, in all 326 samples were cultured. The SM009 S. marcescens could only detected between the two outbreak periods from a water tap, as mentioned before.

Although the source of the outbreak could not be identified during the outbreak periods, data suggest that carrier neonates and infants might be responsible for the persistence of the epidemic strain at the department.

We collected clinical data about 12 *S. marcescens* epidemic and two sporadic BSIs. Median age of the infants was 37 days (range 5-165 days). Median gestational age was 33.5 weeks (range 24-37 weeks), median birth weight was 2070 g (600-3450 g). Five neonates (36%) were extremely low birth weight (ELBW) neonates. Three of the 14 cases (21%) had signs of neuroinfection that accompanied the sepsis, in two cases the pathogen could be isolated from the cerebrospinal fluid as well, besides blood culture. Importantly, only one of the cases with neuroinfection was an ELBW neonate. Twenty-eight day mortality was 29%; three of the four ELBW survived the infection. In the cases that died severe underlying diseases were observed such as intraventricular hemorrhage, jejunal atresia, and trachea-esophageal fistula.

CONCLUSIONS

1. To our knowledge our report is the only multicenter study from the last decades that investigated both microbiological and clinical aspects of pediatric Gram-negative sepsis in Hungarian children.

2. The pathogens studied in the survey caused mostly nosocomial and healthcare associated infections. Great majority of the children suffering from Gram-negative sepsis had severe underlying diseases, the greatest patient groups were ICU patients, hematology-oncology patients, and infants treated in the neonatal intensive care unit.

3. The pathogen distribution in our study was similar to previous reports; most frequent bacteria were *Enterobacteriaceae* (*E. coli*, *Klebsiella spp*., *Serratia marcescens*, *Enterobacter spp*.) and *Pseudomonas aeruginosa*. Pathogens

associated with highest mortality were *P. aeruginosa*, *S. marcescens*, *Enterobacter spp*., and *S. maltophilia*.

4. Rates of antimicrobial resistance are high, multidrug-resistant pathogens could be detected in 33.6% of bloodstream infections. Cefotaxime, meropenem, ceftazidime, and piperacillin/tazobactam showed relatively good efficacy, and of note, rates of aminoglicoside resistance (especially amikacin) were low. In patients treated in hematology-oncology meropenem and ceftazidime were more effective than piperacillin/tazobactam. Multidrug-resistance is associated with age (in older children MDR rates are higher), and with the ward at which the child is treated (ICU and hematology-oncology patients are more prone to have an MDR pathogen). Stem cell transplantation patients, neutropenic patients, and patients with polymicrobial infections are of particularly high risk of acquisition of a MDR Gram-negative pathogen. Among frequent pathogens *E. coli*, *P. aeruginosa* and *Enterobacter spp.* had the highest rates of multiresistance. Prior cephalosporine treatment was a risk factor for MDR infections, and MDR rate was higher in children with multiple organ failures. In conclusion, for these patient groups – considering the high rates of multidrug-resistance – broad spectrum antibiotics (e.g. broad spectrum beta-lactams such as meropenem), or even combination of antibiotics as empiric therapy (e.g. meropenem + amikacin) could be suggested.

5. Mortality rates were similar to the results of previous surveys originating from other countries. Importantly, sepsis-related mortality was much lower than all-cause mortality, suggesting that underlying diseases may play a very important role in the prognosis of children suffering from Gram-negative sepsis. Children treated in the ICUs and hematology-oncology wards had higher mortality rates than infants treated in the NICU. According to our results ICU care, transfusion, stem cell transplantation, urinary catheterization, parenteral nutrition and septic shock are risk factors of mortality in Gram-negative pediatric sepsis. Multiresistance did not affect mortality significantly, although there was a trend suggesting that patients suffering from MDR infection are more prone to die.

6. The survivors of Gram-negative pediatric sepsis (in terms of 28-day survival) have questionable long-term prognosis, because a greater part of these children needed further hospital treatment after closure of data collection. Studies addressing the long-term follow up of these children could answer the questions about long-term prognosis, quality of life and life expectancies of children surviving Gram-negative sepsis.

7. During the *S. marcescens* outbreak we observed that a virulent epidemic strain is able to persist for long periods in asymptomatic carriers that may serve as a source of persistent or recurrent outbreaks. Circulation of the epidemic strain between patient of wards and institutions always carries the risk of a new shub of an outbreak. Besides environmental sources such as drugs, infusions, parenteral nutrition solutions, mechanical ventilators and hand of healthcare worker, carrier patient should be considered as source of epidemic infections. Surveillance cultures and molecular epidemiology methods may play an important role in the early identification of alarming pathogens.

8. In line with the medical literature we observed that neonatal *S. marcescens* infections carry a high risk for development of neurological complications such as meningitis and brain abscess. Although the epidemic strain was susceptible to most antimicrobial agents, it is known that that this species may have a lot of inducible antimicrobial resistances. In conclusion, these neonatal invasive infections should be treated with broad spectrum antibiotics with good penetration to the cerebrospinal fluid. In suspect of neurological complications, early radiologic workup (e.g. brain MRI for abscess) is advisable.

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