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(RESEARCH ARTICLE)

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Outbreak of nosocomial sepsis in NICU by multidrug-resistant *Klebsiella pneumoniae*: Diagnostic challenges

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Abstract

Purpose: Nosocomial infections are a serious problem in neonatal intensive care units (NICU) – they are one of the main causes of morbidity and mortality among preterm newborns.

Material/methods: We present a prospective clinical-epidemiological study of an outbreak of 7 cases of culture-proven multidrug-resistant (MDR) *Klebsiella pneumoniae* late onset sepsis in a period of 2 months in a tertiary level NICU in Pleven, Bulgaria. The risk factors, early clinical presentation, laboratory changes, course of infection, treatment, and outcome were evaluated.

Results: Three of our patients were term infants, the other four patients were preterm aged from 25 to 36 gestational weeks. The most possible routs of transmission of the infection were: venous lines, mechanical ventilation, tube feeding. All of the mentioned were present in one extremely immature patient.

Early clinical signs included skin color changes, abnormal muscle tone, respiratory failure. Laboratory tests taken at the time of onset revealed leucopenia and/or thrombocytopenia in a half of the cases and usually C-reactive protein remained normal, but in all cases the I/T ratio increased significantly. The clinical course varied from fulminant deterioration to easily controlled condition. Treatment with Meropenem was started at the time of clinical suspicion of infection, according to the antibiogram of the first cases. No fatal outcome was observed.

Conclusion: Main causes for the outbreak of hospital acquired infection were overcrowding of the NICU, admission of patients from lower-level hospitals, insufficient working staff, especially midwifes. The early diagnosis and proper antimicrobial treatment were crucial for the favorable outcome.

Keyword: Outbreak; Nosocomial Sepsis; Neonate; *Klebsiella pneumoniae*

1. Introduction

Nosocomial infections (NIs) or hospital-acquired infections (HAIs) are infections, acquired in various medical settings where patients receive health care. They develop after the 72nd hour of hospitalization and may be presented clinically in varying degrees of severity. The immunological characteristics of newborns, especially the premature, and the performed invasive diagnostic and therapeutical procedures make the Neonatal Intensive Care Unit (NICU) a particularly risky department for the development of nosocomial infections. NIs are one of the main causes of morbidity and mortality among preterm newborns.

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The overall incidence ranges from 8.9 to 62 infections per 1,000 patient-days, or 6 to 25% of the NICU population. Infection rates vary for each country, according to its level of socio-economic development and the competence of the neonatal care. The latest systematic review and meta-analysis, published in January 2021, found that the neonatal sepsis incidence (early and late onset sepsis) on a global level is 2,824 cases per 100,000 live births and the mortality is 17.6%. According to the Global Burden of Disease (GBD), there are 1.3 million annual incident cases of neonatal sepsis and other infections. Premature and low birth weight infants were mainly affected. Approximately one-third of infections were culture-proven, caused by *Staphylococcus aureus* and *Klebsiella* spp. [1].

Not much data is available on the HAIs in Bulgarian NICUs. An epidemiological surveillance from 2020 carried out at St. George University Hospital in Plovdiv on 507 hospitalized newborns, estimated that the incidence was 9.5% per 1,000 patient-days, presenting as ventilator-associated pneumonia (VAP) (67.27%), bloodstream infection (23.64%) and conjunctivitis (9.09%). Major pathogens were Gram-negative such as *Klebsiella pneum*oniae, *Escherichia coli*, *Pseudomonas aeruginos*a and *Acinetobacter baumannii* [2].

Klebsiella pneumoniae is an opportunistic pathogen and a leading cause of late-onset neonatal sepsis (LOS) in lower-middle- and lower-income countries. It is a Gram-negative, non-motile, and usually encapsulated bacillus of the *Enterobacteriaceae* family [3]. The bacteria is characterized by high virulence, ability to colonize infants and to survive on skin and in inanimate environment, and ability to acquire antibiotic resistance determinants. Main reservoirs in the NICU are the gastrointestinal tract of premature infants and the hands of medical staff. Increased use of cephalosporins results in growing Carbapenem resistance and microbial hypervirulence [4].

2. Material and methods

We present a prospective clinical-epidemiological study of an outbreak of 7 cases of culture-proven hospital-acquired sepsis in a period of 2 months (May-June 2022) in a tertiary level NICU in University hospital "Dr. Georgi Stranski", Pleven, Bulgaria. Target group were patients with septicemia, diagnosed by clinical features and positive blood culture result, where *Klebsiella pneumoniae* was the proven causative agent. The isolates were identified by BACTEC automated blood culture system and routine antimicrobial susceptibility testing was performed. Detailed data was collected from the medical documentation, concerning the risk factors, early clinical presentation and laboratory changes, course of infection, treatment and outcome. Descriptive analysis and Microsoft Excel were used.

3. Results

For the studied period, 50 patients were treated in the NICU for more than 72 hours, 14 of which weighed under 1500 g. *Klebsiella pneumoniae* sepsis was registered in seven patients, from which four were preterm, two were under 1500 g and one was under 1000 g. Male gender prevailed (5/7). The mean time of onset of infection was 6.7 days after birth (Table 1).

Table 1 Demographic analysis of the study population

Characteristics		**n**
Preterm delivery		4
Birth weight (g)	<2500	1
	<1500	2
	<1000	1
Gender F/M		2/5
Caesarian section		1
Outborn		2
Day of onset after birth (mean value)		6.7

*Outborn – born in another clinic and transported to the NICU

The possible sites at risk for invasion by microorganisms were discussed: venous accesses (2 central and 5 peripheral venous lines) – seven patients, most of them receiving parenteral nutrition; mechanical ventilation – one patient on

invasive and two patients on non-invasive ventilation; tube feeding was a risk factor in four of the cases. All of the mentioned possibilities were present in one extremely premature patient. Main risk factors from the environment for this period were overcrowding, admission of patients from lower-level hospitals, and insufficient working staff, especially midwifes (Table 2).

Table 2 Risk factors

Risk factors	Number of affected patients
Mechanical ventilation	3
Central venous catheter	2
Peripheral venous line	5
Parenteral nutrition	6
Gastric tube	4
Hypotrophy	1
Prematurity	4
Bioproduct transfusion	1
Congenital anomaly	0
Chronic cardiac/respiratory disease	0

Early clinical signs included respiratory failure, lower muscle tone, tachycardia, and changes in skin color. The last patient showed no deterioration in the general condition; the infection was detected by laboratory tests and early treatment was successful (Fig. 1) (Table 3).

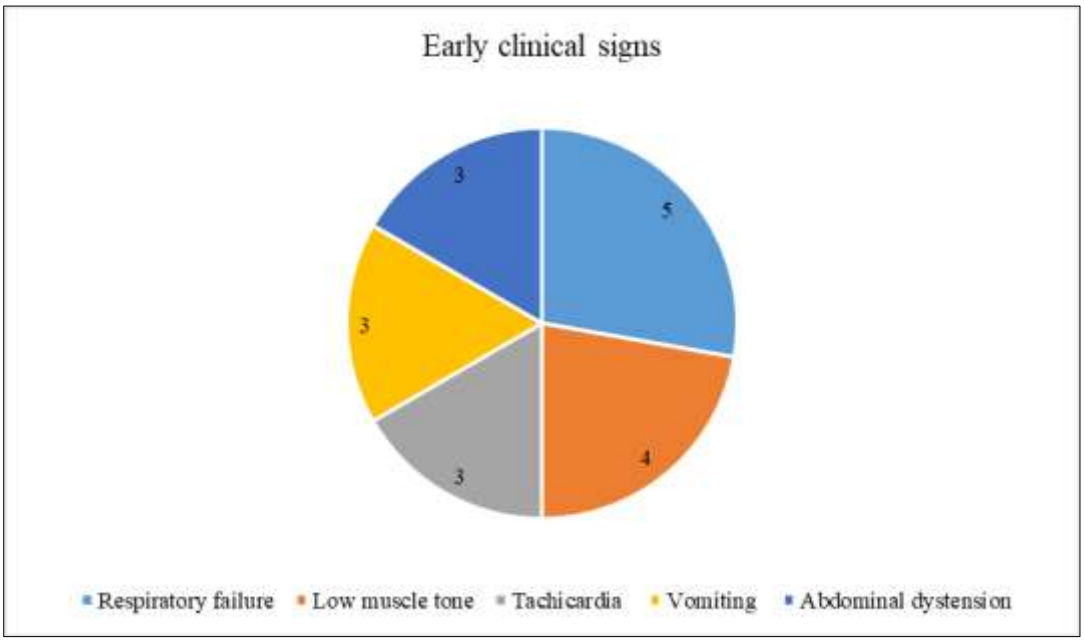

Figure 1 Prevalence of early clinical signs

Table 3 Early clinical signs of infection

Early clinical signs	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Pallor, grey skin					+	+	
Tachycardia		+	+		+		
Respiratory distress	+		+	+	+	+	
Purulent bronchial secretions	+			+			
Temperature instability		+			+		
Abdominal distention	+		+		+		
Vomiting	+		+		+		
Low muscle tone		+	+	+	+		
Irritability, seizures						+	
Jaundice		+			+	+	

Laboratory tests taken at the time of onset showed leucopenia (WBC < 5 x 10^9/L) and/or thrombocytopenia (PLT < 150 x 10^9/L) in most of the cases and usually C-reactive protein was not abnormal (<5 mg/L), but in all cases the immature-to-total neutrophil (I/T) ratio was high (>0.2). In two of the cases necrotizing enterocolitis (NEC) was suspected and fecal calprotectin was evaluated; the elevated value supported the diagnosis (>50 μg/mg) (*Fig.* 2) (Table 4).

Table 4 Laboratory data changes and time of change. Day 1 is the day of clinical onset of the infection and microbiological probe being taken; laboratory changes are followed by the third day of onset of infection.

Laboratory data	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Low WBC (<5x10^9/L)	Yes Day 1	Yes Day 1	Yes Day 1	No	Yes Day 1	No	No
High I:T ratio (>0.2)	None	Yes Day 1	Yes Day 1	Yes Day 1	Yes Day 1	Yes Day 1	Yes Day 1
High WBC (> 21 x10^9/L)	No	No	No	No	No	Yes On 2. day	Yes Day 1
Anaemia	Yes On 2. day	No	No	No	No	No	No
Low platelet count (< 150 x10^9/L)	Yes On 2. day	Yes Mild Day 1	Yes Severe Day 1	No	Yes On 3. day mild	Yes Severe Day 1	Yes Severe Day 1
CRP (> 5 mg/L)	Yes On 2. day	Yes On 3. day	Yes Day 1	Borderline	Yes On 3. day	Yes Day 1	Yes Day 1
Fecal calprotectin (>50 μg/mg)	Borderline	None	None	None	Yes Day 1	None	None

*Mild thrombocytopenia refers to 100-150 x 10^9 /L; severe thrombocytopenia <100 x10^9/L.

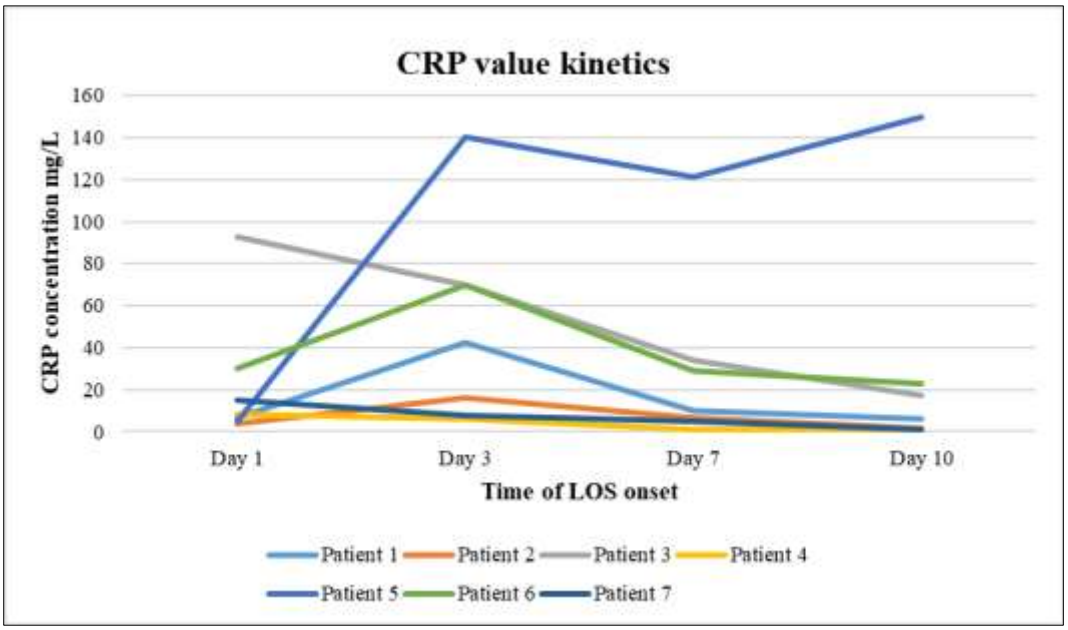

Figure 2 Changes in CRP value in time. Day 1 is the day of clinical onset of the infection and microbiological probe being taken. Before this day all patients had normal value of CRP. Only 3 of the patients have significantly high value >5 mg/L at the time of clinical suspicion of LOS

The clinical course varied from fulminant deterioration (need of mechanical ventilation, blood pressure optimization, blood product transfusion) to easily controlled condition. Two of the infants had serious complications – Patient 5 developed necrotizing enterocolitis (NEC) and periventricular leukomalacia with long-term impairment; patient 6 was diagnosed with endocarditis, which is rarely seen in *Klebsiella* sepsis but another probe showed also *Staphylococci* and the causative agent was not exactly known.

Treatment with Meropenem was started at the time of clinical suspicion and total blood count changes, according to the antibiogram of the first cases. Some of the blood cultures showed susceptibility to Cefoxitine, which is not used in our clinic. Seven episodes of sepsis were confirmed microbiologically from 6 blood cultures, 3 tracheal aspirates, 2 tube tips and 1 central blood line tip, all showing multi-drug resistant *Klebsiella pneumoniae*. No fatal outcome was observed (Table 5).

Table 5 Antibiotic susceptibility of the isolated strain Klebsiella pneumoniae

Antibiotic/Susceptability	**All**	**S**	**I**	**R**
Ampicillin	11			11
Amoxicillin/clavulanic acid	11			11
Piperacillin	11			11
Piperacillin/tazobactam	11			11
Cefazolin	9			9
Cefuroxime	9			9
Cefoxitin	9	8		1
Cefotaxime	9			9
Ceftriaxon	9			9
Ceftazidime	11			11
Cefepime	11			11
Cefoperazone/sulbactam	9		2	7

Imipenem	11	11		
Meropenem	11	11		
Gentamicin	11			11
Amikacin	11			11
Tobramycin	11			11
Ciprofloxacin	11			11
Levofloxacin	11	2	7	2
Doxycycline	6	6		
Tigecycline	5	5		
Trimethoprim/sulfamethoxazole	3			3
Colistin	11	11		

4. Discussion

Klebsiella pneumoniae has been identified as one of the leading causes of infectious outbreaks in neonatal intensive care units (NICU). Newborns are more susceptible to this opportunistic pathogen due to their immature immune systems, low birth weight and frequent use of invasive devices and antibiotics [5]. A German study reported that nearly 50% of *Klebsiella pneumoniae* outbreaks occurred in the NICUs and were controlled using a mixture of different infection control measures [6]. The importance of proper antibiotic policies, less invasive procedures, hand disinfection / washing before and after patient treatment, and isolation precautions are emphasized by many authors [7].

Efforts are being made in the area of early identification of late-onset sepsis. There is a great interest and need for an easy-to-use and accurate scoring model. Some of these scoring systems consist exclusively of clinical variables or laboratory variables, while others use only risk factors and other combinations of these types of parameters [8]. A 2023 study proposed a sepsis prediction score for the early diagnosis of late-onset sepsis of the newborn, including clinical and laboratory abnormalities, but there is still no universally accepted sepsis calculator, as for the early sepsis of the newborn. Multicenter studies on risk factors, clinical and laboratory abnormalities, and the correct combination of these are needed to create a more accurate LOS risk scale. Despite the small number of patients, typical clinical manifestations and laboratory abnormalities were also presented in our cases, which enabled us to identify early the newborns at risk and, together with other anti-epidemic measures, to limit the outbreak of *Klebsiella* spp. HAIs in the clinic.

It is well known that the incidence of LOS is inversely related to birth weight (BW) and gestational age (GA). According to a review by Dong et Speer, analyzing data from studies on over 10,000 newborns, 36.3% of patients with GA <28 gestational weeks (GW) had at least one episode of LOS, compared to 29.6%, 17.5% and 16.5% respectively in babies, born at 29-32 GW, late preterm infants (33-36 GW) and term infants [7].

Besides prematurity, other well-documented risk factors for LOS include: use of invasive interventions such as mechanical ventilation and intravascular catheterization, failure of early enteral feeding with breast milk, prolonged parenteral nutrition, surgical interventions, and underlying respiratory and cardiovascular diseases [9].

In our study, unexpected fact was that three of the patients were term infants, having a peripheral venous line as one and only risk factor. They were born with an underlying pathology – congenital infection, treated with first line antibiotics (ampicillin, gentamicin) and stayed with their mothers before the diagnosis of HAI. The midwife attending to these patients was not working with patients, requiring intensive care but the rooms for antibiotic, infusion and formula milk preparations were shared for all patients. All other preterm infants were formula fed through a gastric tube. Surprisingly, mechanical ventilation takes the 5th place in the frequency grading of risk factors for our patients.

Although we located the connecting places between all cases of *Klebsiella* infection, the regulatory authorities for HAIs control from the health inspectorate didn't isolate the bacteria from swabs and probes, taken from staff's hands or surfaces, ventilators etc. Nevertheless, we installed UVC lamps for additional disinfection in these two common rooms for preparations.

There are variations in the clinical characteristics of infection by Gram-negative and Gram-positive microorganisms. An investigation of a similar outbreak of *Klebsiella* sepsis in Italian NICU by Ampicillin/Piperacillin resistant *Klebsiella* strain showed that all cases presented with symptoms like tachypnea, hypotension, hypothermia and feeding intolerance [5]. This correlates with the early symptoms in most of our patients.

Klebsiella spp. cause predominantly sepsis, but it is a known causative agent of respiratory tract infections, urinary tract infections (UTIs), invasive liver abscesses, even endophthalmitis, and endocarditis [4].

In our study, patient 6 is the first case of endocarditis, associated with the isolation of *Klebsiella*, with a protracted course and discrete symptoms such as irritability, pallor, heart murmur, laboratory abnormalities. The diagnosis was not expected because invasive procedures such as central venous line and operative interventions were not presented in this patient.

The other rare diagnosis was found in patient 5 who developed NEC and underwent surgery for intestinal perforation. Simultaneously with the deterioration, a blood culture was taken and *Klebsiella* was isolated. NEC is a heterogeneous disease with incompletely understood pathophysiological mechanisms, part of which are bacterial invasion and inflammation of the intestine. Usually, microbiological samples remain sterile in a "classic" NEC. In the literature, we found little proof of outbreaks of NEC, associated with a certain causative agent. Gregersen and al [10] published six patients with severe NEC and proven *Klebsiella pneumoniae* bacteriemia with extended spectrum beta-lactamase production. All of the patients developed severe thrombocytopenia.

Furthermore, Gram-negative LOS, fungemia or polymicrobial LOS have significantly higher rates of certain clinical manifestations, including septic shock, disseminated intravascular coagulopathy, leukopenia, thrombocytopenia, anemia and metabolic acidosis than Gram-positive LOS [9]. Thrombocytopenia also prevailed in our cases, and in Patient 7 it was even the only sign of infection, without any early significant deviations in the clinical condition. Leukopenia is also indicative of severe infection, while C-reactive protein is usually still within reference limits when sepsis is suspected. This is expected because it is said to be a "late marker" of inflammation and takes at least 24 hours for the serum concentration to rise. Although rarely mentioned in the literature, we found that an elevated I/T index is a reliable marker for the early diagnosis of systemic infection.

The thorough investigation of patients at risk, clinical picture and laboratory abnormalities helped in early initiation of the correct antibiotic therapy. In our case, it was a multi-resistant strain, as in all cases the only available antibiotic to which *Klebsiella* remained sensitive was Meropenem. No detailed testing of microorganisms was available in our laboratory to study the strain's qualities and resistance mechanisms.

5. Conclusion

According to our data main causes for the outbreak of hospital acquired infection were overcrowding, admission of patients from lower-level hospitals, insufficient working staff, especially midwifes. The most important risk factors were parenteral nutrition and peripheral venous line placement. First clinical signs were respiratory distress and low muscle tone. The early diagnosis and proper antimicrobial treatment were crucial for the favorable outcome.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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