HOSPITAL OUTBREAK INFECTIONS CAUSED BY *SERRATIA MARCESCENS* IN THE NEONATAL INTENSIVE CARE UNIT - CASE REPORT

OHNISKO NEMOCNIČNEJ NÁKAZY VYVOLANÉ SERRATIA MARCESCENS NA NOVORODENECKEJ JEDNOTKE INTENZÍVNEJ STAROSTLIVOSTI – KAZUISTIKA

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ABSTRACT
Introduction: Serratia marcescens is an opportunistic human pathogenic microbe often involved in hospital outbreaks in neonatal intensive care units (NICU). It presents a serious problem, especially in preterm infants. There it can be associated with a life-threatening manifestation of infection.
Aims: The purpose of the review is to analyse the hospital outbreak caused by multidrug-resistant *S. marcescens* in the NICU.
Material and Methods: We describe an outbreak in which *S. marcescens* occurred in 3 patients that lasted from October 10 (first positive nasal swab in neonate) to November 14 (discharge of the last patient). The patients are presented as case reports in the context of the results of swabs collected from the patients and from the environment, as well as the spatial organization of the NICU.
Results: In the first case, the infection had an asymptomatic course, but in the second and third it developed in severe clinical forms, including sepsis and meningoencephalitis. The diagnosis was based on clinical manifestation and positivity of swabs from respiratory tract, umbilical venous catheter, and blood. The environmental samples were negative.
Conclusion: The specific source of infection cannot often be identified. Direct transmission of the infection from the first patient could not have occurred because they were not present in the NICU at the same time. The transmission probably occurred indirectly through the water sinks and the hands of the staff. To contain the spread of infection, control measures in the environment (disinfection and replacement of sink drains) and early identification of colonized/infected newborns immediately after the first positive swab should necessarily be implemented independently of the clinical manifestation. From a preventive aspect, it is important to properly adjust the spatial equipment of the NICU with the specification of maximum patients and the possibility of regular replacement of the water drains.

Key words: *Serratia marcescens*. Outbreaks. Prematurity. Hospital infection.

ABSTRAKT
Úvod: *Serratia marcescens* je oportúnny ľudský patogén, ktorý je častým pôvodcom nozokomiálnych nákaz na neonatologických jednotkách intenzívnej starostlivosti (NICU). Predstavuje vážny problém, najmä u predčasne narodených novorodencov, kde infekcia môže mať život ohrozujúci priebeh.
Cieľ: Cieľom práce je analyzovať nemocničnú epidémiu spôsobenú multirezistentným kmeňom *S. marcescens*, ktorá prebehla na NICU.
Materiál a metódy: Jednalo sa o epidémiu postihujúcu 3 pacientov, ktorá prebiehala od 10. októbra 2019 (prvý pozitívny výter z respiračného traktu novorodenca) do 14. novembra 2019 (prepustenie posledného prípadu). Jednotlivé prípady uvádzame ako kazuistiky v kontexte výsledkov kultivácií sterov odobratých od pacientov aj z prostredia ako aj priestorového usporiadania neonatologickej jednotky intenzívnej starostlivosti.
Výsledky: U prvého prípadu mala infekcia asymptomatický priebeh, avšak u druhého a tretieho prípadu sa rozvinula do ťažkej klinickej formy vrátane sepsy a meningoencefalitídy. Infekcia bola diagnostikovaná na základe klinického obrazu a pozitivity vzoriek odobratých z respiračného traktu, umbilikálneho venózneho katétra a krvi. Stery z prostredia boli negatívne.
Záver: Primárny zdroj infekcie *S. marcescens* sa často neidentifikuje. K priamemu prenosu nákazy od 1. pacienta nemohlo dôjsť vzhľadom na to, že sa v NICU nenachádzali naraz v jednom čase. K prenosu došlo nepriamo, pravdepodobne prostredníctvom vodovodných sifónov a rúk zdravotníckeho personálu. Pre zastavenie šírenia nákazy je kľúčové vykonanie opatrení v prostredí (dezinfekcia a výmena vodovodných sifónov) a aktívne vyhľadávanie kolonizovaných/infikovaných novorodencov bezprostredne po prvom pozitívnom kultivačnom náleze nezávisle od klinického obrazu. Z hľadiska prevencie je dôležitá správna úprava priestorového vybavenia NICU so stanovením maximálneho počtu pacientov a možnosťou pravidelnej výmeny vodovodných sifónov.

Kľúčové slová: *Serratia marcescens.* Epidémia. Prematurita. Nozokomiálna nákaza.

INTRODUCTION

Serratia marcescens ranges among rod-shaped bacteria in the family *Enterobacteriacae*. It is an important opportunistic pathogen responsible for hospital infections, particularly in immunosuppressed premature newborns hospitalized in neonatal intensive care units (NICUs). It is responsible for approximately 12 % of hospital epidemic outbreaks [1]. According to the findings of the European multicenter study focused on pediatric hospital infections, the *S. marcescens* caused up to 15 % of culture-positive infections in the NICUs [1-5]. The European Center for Disease Prevention and Control (ECDC) in its 2016 report considers *Serratia spp.* as the sixth most common strain isolated in hospital-acquired pneumonias in patients in the intensive care unit in Europe [2, 4, 6]. According to recent data, late hospital infections caused by *S. marcescens* strains occur in 0,9 – 2.3 cases per 1000 preterm infants and are associated with a significantly higher case-fatality rate [3, 6, 7]. *Serratia marcescens* – caused infections account for almost 8% of hospitalized newborn deaths in the NICU [1].

Serratia spp. can grow at temperatures from 5 to 40 centigrades and in media with pH values between 5 and 9. It survives in humid environments and most commonly can be found in water siphons as part of a biofilm where it can survive several years [2, 6, 8-10]. Moreover, it can also be found on a surface of taps, bathtubs, incubators, in tap water, inside air conditioning systems and breast pumps. It can also contaminate breast milk, injectable drugs, parenteral nutrition, liquid soaps and shampoos, as well as disinfectants [1, 2]. Therefore, it can easily survive and spread in the hospital environment resulting in epidemic outbreaks. An infected newborn is the most common source of infection, especially if the agent is located in its respiratory and gastrointestinal tract [1, 3, 4, 6]. The agent is transmitted mainly through the hands of healthcare workers. The high occupancy of the NICU and the low ratio of nurse to patient significantly increase the risk of transmission [2]. Commonly occurring antibiotic resistance, namely to aminopenicillins, combined aminopenicillins with inhibitors of beta-lactamase , ureidopenicillins, as well as third-generation cephalosporins, present another problem related to the management of outbreaks caused by *S. marcescens.* Furthermore, resistance to gentamicin and tobramycin has also been described [1, 11]. Intensive decontamination of surfaces can also lead to the development of resistance to disinfection agents [2, 6, 12].

In the case study, we analyse local epidemic outbreak in the NICU of the 3rd level regional hospital and based on the findings, we propose preventive approaches regarding high-risk premature newborn treatment, as well as changes in the spatial arrangement to minimize the risk of further outbreaks of *S. marcescens* infections.

Analysis of the epidemic outbreak in the NICU in October 2019 caused by *Serratia marcescens*

In 2019, the NICU of the Neonatology Clinic had a capacity of 6 intensive care beds and provided acute resuscitation and intensive care to critically ill newborns born within Region's catchment area. The average occupancy was 12 newborns per month.

The NICU consisted of two interconnected rooms with separate entrances from the hallway and the administrative room. Two nurses (one nurse per 3 patients), one head shift nurse, and two physicians worked in the unit during 8-hour shifts. The spatial arrangement of sinks, incubators, a table for preparing medicines, and other parts of the rooms is shown in Figure 1.

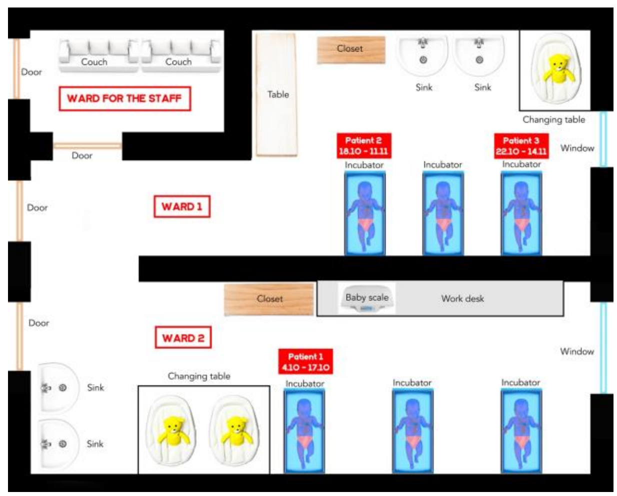

Figure 1 Spatial arrangement of the NICU showing the location of incubators and sinks

The local epidemic outbreak that broke out in October 2019 included three cases of positive cultivated *S. marcescens*. One of them showed a serious course with meningoencephalitis and the development of a brain abscess.

The first case was hospitalized in room No. 2 from 4 October 2019 to 17 October 2019. The patient was born in the 26th gestational week with a weight of 830 g. He was referred to the NICU at the age of 5 weeks with ventilation support through a high flow cannula needed for 23 % oxygen and the general condition stabilized. The initial ear swab was negative. Later, in the respiratory tract (nasal)

Table 1 Resistance to phenotype antibiotics in cultivated strains of *S. marcescens*

Antibiotic sensitivity	Pacient 1 (the both swabs)	Pacient 2	Pacient 3
Ampicillin	R > 32	R > 32	NA
Ampicillin-Sulbactam	R > 32	R > 32	R >= 32
Piperacillin-Tazobactam	S 1	S 0.5	S 8
Cefuroxime	R > 32	R > 32	NA
Cefotaxime	I 2	S 0.25	R >= 64
Ceftazidime	S 0.5	S 0.25	R 32
Cefoperazone-Sulbactam	S 1	S 0.5	NA
Cefepime	S 1	S 0.25	I 4
Ertapenem	S 0.03	S 0.03	S <= 0.12
Meropenem	S 0.12	S 0.12	S 1
Gentamicin	S 0.5	S 0.5	S 2
Tobramycin	S 0.5	S 0.5	NA
Amikacin	S 1	S 1	S 4
Tigecycline	R 4	S 0.5	NA
Ciprofloxacin	R 2	S 0.12	S <= 0.25
Tetracycline	R > 16	R >16	NA
Colistin	R > 8	R > 8	R > 16
Trimethoprim-Sulfamethoxazole	I 4	S 0.25	S 1
Fosfomycin	NA	NA	R >= 16

Legend: *NA – sensitivity has not been determined, R – resistance, S – sensitivity, I – intermediate sensitivity

swab taken on 10 October 2019 multiresistant *S. marcescens* was found. A detailed overview of the phenotypic antibiotic resistance of the cultivar strains is shown in Table 1. Additionally, another swab taken from the respiratory tract (the same place) on 16 October 2019 was positive. The child did not show any clinical signs of infection.

The second case was hospitalized in room No. 1 in the incubator next to the door from 18 October to 11 November 2019. The patient was born spontaneously by vertex birth in the 31st gestational week after complete corticoid preparation with a birth weight of 1,060 g. After 72 hours of hospitalization, the clinical condition suddenly worsened with the development of early sepsis and meningitis. Bloodstream infection required complex treatment with the administration of antibiotics (ampicillin, gentamicin), thromboconcentrate, immunoglobulins and dopaminergic support due to blood pressure instability. The clinical condition also required the use of artificial pulmonary ventilation and the insertion of a central umbilical venous catheter due to the necessity of using highly concentrated drugs. Insulin was administered continuously due to metabolic disruption caused by hyperglycemia. In the blood culture taken on 21 October 2019, *Escherichia coli* bacteria with good sensitivity to antibiotics were detected. On 23 October, the umbilical venous catheter replaced and *E. coli* with good antibiotics, as well as *S. marcescens* resistant to ampicillin, ampicillin sulbactam, cefuroxime, tetracycline, and colistin were cultured from its centrally inserted end (Table 1). The double antibiotic combination was given for the next 21 days. During treatment, the clinical condition improved, and the inflammatory parameters decreased. In further respiratory tract swabs, including the intubation cannula, *S. marcescens* was not found.

The third case was hospitalized in room No. 1 next to the window from 22 October to 14 November 2019. The child was born at the 34th gestational week by acute caesarean section due to severe HELLP syndrome in the mother. Due to the development of tachydyspnea in the early postpartum period, continuous positive airway pressure ventilator support (CPAP) was initiated. Also, an epicutaneous central venous catheter was inserted due to poorer tolerance of oral intake and partial parenteral nutrition was administered through it. On 28 October (8. day of hospitalization) the clinical condition suddenly worsened with febrility and the development of a septic condition. Treatment with a double combination of antibiotics (cefotaxime, gentamicin) was started due to a significant elevation of inflame-

Table 2 Risk factors for hospital-acquired infection in patients

Risk factors	Case 1	Case 2	Case 3
Gestational age	26	31	34
Birth weight	830g	1490g	2000g
Invasive procedures	ALV, UVC, EVC	UVC, EVC, CPAP	CPAP, EVC
Prophylactic application of antibiotics	yes	no	no
Ventilation	CPAP, HFNC	ALV, CPAP	CPAP
Length of hospital stay (day of the positive swap)	78 (42.)	25 (5.)	30 (8.)
Sectio caesarea	yes	no	yes
Cardiopulmonary resustitation	yes	no	yes

Legend: ALV - Artificial lung ventilation, UVC - Umbilical venous catheter, EVC - Epicutaneous venous catheter, CPAP Continuous positive airway pressure, HFNC - High flow nasal cannula

Table 3 Timeline of hospital stay and positive cultivation findings. (The arrow shows the 14-day interval between the first positive nasal swab and the development of severe sepsis).

	10.10	12.10	14.10	16.10	18.10	20.10	22.10	24.10	26.10	28.10	30.10
Patient 1 NICU 4-17.10	**+ Nasal swab**			**+ Nasal swab**	-	-	-	-	-	-	-
Patient 2 NICU 18.10.-11.11	-	-	-	-	*	**E.coli Early sepsis**		**+ UVC**			
Patient 3 NICU 22.10-14.11	-	-	-	-	-	-	*			**+ HK**	**+ CSF**

Legend: UVC – positive umbilical drain swab, * date of birth, HK – positive hemoculture, NICU – neonatal intensive care unit, CSF – biochemical positivity of cerebrospinal fluid, + positive swab, ■ – hospitalization in NICU, ⇔ – interval between the first positive swab and development the severe sepsis

matory parameters, thrombocytopenia and leucopoenia. Blood plasma and dopaminergic support were administered due to hypotension and hypoperfusion of the peripheral blood stream. Convulsions that did not respond to combined anticonvulsant therapy developed within 12 hours after the appearance of clinical symptoms. Therefore, the patient was intubated and connected to artificial pulmonary ventilation, and the second epicutaneous central venous catheter was inserted. Inflammatory parameters continued to increase despite treatment, so after 12 hours the treatment was changed to meropenem and gentamicin. Subsequently, the condition began to gradually stabilize, and the inflammatory parameters decreased by 12 hours. Thromboconcentrate was also administered due to thrombocytopenia. On 30 October 2019, a brain ultrasound examination showed a hyperechoic frontoparietal focus on the right cerebral hemisphere. However, despite treatment, the ultrasound of the brain progressed, and the four-chamber hydrocephalus developed. The follicle of the purulent contents and a biochemical picture of purulent meningitis were present in the cerebrospinal fluid, but *S. marcescens* was no longer found. From the original hyperechoic focus in the right hemisphere, two frontotemporal-parietal hyperechoic foci of size 4 x 4 cm with central porencephalies were formed, and at the same time the midline structures were displaced due to oppression of the right lateral ventricle. In the blood culture taken on 28 October 2019, *S. marcescens* resistant to ampicillin, ampicillin, sulbactam, and the third generation cephalosporins was found. Swabs taken from the respiratory tract, as well as control blood cultures taken weekly, were negative.

In view of this outbreak, we launched an investigation in November 2019 to find reservoirs and transmission factors in the environment. In total we took 23 samples (faucet, bathtub, washbasin 3x, table, sheet, clean napkin, clean shirt from laundry, sterile napkins, liquid soap, parenteral nutrition, incubator 3x, humider, hand cream, baby scale, powdered baby milk, water from milk heater, air filtration in incubator, clean stethoscope, cold water from barrel, hot water from barrel). Of these, the smears were positive from the bathtub in room No. 1

(*Enterobacter spp.*, *Pseudomonas aeruginosa* and *E. coli*), from the sink in room No. 2 (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Clostridium perfringens* and *Burkholderia spp*.). *S. marcescens* was not detected in either environment or respiratory tract samples in hospitalized patients. We subsequently intensified restrictive hygiene measures. They consisted of restriction of work on NICU and repeated decontamination of surfaces, including siphons.

The staff was repeatedly re-trained in hand hygiene and an observation program has been introduced to determine the compliance of hand hygiene according to the WHO. Since then, no further cases of *S. marcescens* infection have appeared. The detailed investigation of each hospital infection in the NICU should be an essential part of the proper operation of the department and the improvement of health care. The outbreak analysis provided arguments to receive financial resources for spatial reconstruction of the NICU, thus improving found insufficiencies and to meet the latest health care standards regarding critically ill newborns. See Table 3 for a timeline of the cases.

DISCUSSION

Epidemiological investigation in the NICU, despite negative cultivation, indicated that a biofilm on the sinks and bathtub sinks in both rooms played the role of possible reservoirs. The fastest transmission from Case 1 to Cases 2 and 3 was not possible since their NICU stays did not overlap. Case 1 had been transferred to different section of the department to an isolation room at the time of the delivery of Cases 2 and 3.

Since an entrance screening of the respiratory tract and GIT was not performed in Case 1 at admission to the NICU, it is not possible to determine whether the patient was transported to us already colonized or became infected in the NICU. Swabs from the throat and nose performed during hospital discharge at the pericentre were negative and no anus swabs were performed. Considering the analysed outbreak, two possible scenarios should be taken into account. The first allows the long-term persistence of *S. marcescens* in the sink siphon with successive infection of all three patients. The second scenario admits the possibility of undetected intestinal colonization of Case 1, which contaminated the sinks leading to infection of Cases 2 and 3. The agent could have been transferred by the hands of healthcare workers or by a contaminated water aerosol during the daily and evening bathing of the child above the bath siphon.

Water siphons rank among the most common reservoirs of *S. marcescens* [6, 13]. The study by Muyldermans et al. pointed out the importance of bacterial biofilms on the surface of siphons as aerosol created during hand washing can lead to *S. marcescens* infection unless proper alcohol hand disinfection is performed before handling with patients [9]. In the study that analysed samples from more than 500 water siphons in neonatal intensive care units, *Serratia spp.* was found in more than 44 % of them [6]. Furthermore, in the prospective study, *Serratia spp.* was repeatedly detected in water siphons more than one year after the first detection, confirming persistent colonization [6, 8].

Considering all the above-mentioned facts, we suppose that the water siphon was the reservoir in our analysed outbreak. The agent was probably transferred to Case 3 by the hands of the healthcare workers either from water siphon or from Case 2 considering the risk of insufficient compliance with hand hygiene. Yeo et al. pointed out in their study that hand hygiene of healthcare workers was generally insufficient and initial compliance reached only 83 % and increased to 95 % after repeated training [3].

The limitation of our epidemiologic investigation in NICU environment was that we were unable to take samples directly from the siphons, as it would be necessary to make the sinks unfunctional and to close the whole room at NICU because of the risk of further contamination. Such restrictions were not possible due to limited capacity reasons. So, we could not detect the biofilm formation, including *S. marcescens*, in the sinks. Moreover, the identification of *S. marcescens* from swabs from a siphon is itself difficult, since more than 20 bacterial species can be found in one sample [2, 9, 10, 14]. The absence of *S. marcescens* in smears may be due to the non-selective culture medium, in which detection is rather difficult [12]. Bourdin et al. pointed out the importance of using selective growing medium containing antibiotics that suppress the growth of other bacteria in the biofilm (for example selective McConkey medium) [6]. PCR diagnostics would also be helpful in the identification of reservoirs of *S. marcescens*, as well as other epidemiologically significant pathogens in the environment. After a positive finding of *S. marcescens*, the exact identification of a genotype is important to understand the spread of infection, identifying possible reservoirs of infection and transmission factors in the environment [3, 9]. Several studies reported a high genotypic diversity after a detailed analysis of *S. marcescens* strains, indicating different sources/ reservoirs of infection in neonatal intensive care units [3, 6, 9, 12]. Unfortunately, the agents found were not further stored in the microbiology laboratory, so we were unable to perform whole genome analysis of cultivated strains to confirm that all patients were infected with the same strain of the agent. Furthermore, at the time given, molecular analysis of isolated strains making possible a better clarification of the spread of the infection was not commonly available in Slovakia. Considering the prevention of hospital outbreaks caused by *S. marcescens*, attention should be necessarily focused on the environmental microbial load in addition to the active search for infected and colonized patients [2, 12]. Considering repressive measures, it is important to replace the water siphons in the given ward. In addition, siphons should necessarily be decontaminated weekly with 10 – 25 % peracetic acid [9, 15].

CONCLUSIONS

Hospital infections represent a significant health care issue. Local outbreaks caused by *S. marcescens* strains in the NICU spread rapidly and often have devastating consequences for patients [2, 12]. Therefore, strict control measures are necessary focused on patient isolation, decontamination processes, and identification of possible reservoirs and routes of environmental transmission as early as immediately after the first detection of multiresistant *S. marcescens*, even in asymptomatic patients. The risk of developing a severe infection in a premature patient is very high. During our analysed outbreak, only 14 days elapsed between the positive finding of multiresistant *S. marcescens* in the respiratory tract of the first patient and the development of severe sepsis in the third patient. More intensified measures aimed at stopping the spread of the infection carried out in this time window, including partial closing of the NICU, would possibly prevent the further spread of the infection. Our analysis, despite the limitations, points out the importance of rapid implementation of control measures in the environment immediately after the first detection of *Serratia* spp. in a neonatal ward regardless of clinical manifestation in hospitalized patients. Our findings present an implication for further research on the issue, namely analysis of factors determining indirect transmission of *Serratia spp.* infections in neonatal wards as a base for recommended standard procedures and guidelines.

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