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Outbreak of nosocomial bacteremias, caused by Enterobacter gergoviae and Enterobacter aerogenes, in the neonatal intensive care unit, case - control study

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ABSTRACT

In this case-control study we describe epidemiological characteristics and evaluate risk factors for bacteremias caused by a rare human pathogen Enterobacter gergoviae, and Enterobacter aerogenes, among neonates in the intensive care unit, under conditions of nosocomial outbreak.

Crude rate of bacteremias was 16 per 1000 admissions. Bacteremias most commonly occurred between 7th to 30th day of hospitalization and were 1.9 times more frequent in males than females. The following risk factors were significantly associated with the development of bacteremias: a) colonization or infection of neonates prior to the onset of bacteremia with Enterobacter spp. (OR=3.4, 95%CI=1.2-9.9) or non-Enterobacter spp. (OR=7.9, 95%CI=1,2-52.5); b) use of antimicrobial drugs most notably ceftazidime (OR = 7.8, 95% CI = 1.6.-38.6), or amikacin (OR = 7.5, 95% CI = 2.8-19.9); and c) invasive interventions: mechanical ventilation (OR = 4.7, 95% CI = 1.6-13.5), umbilical catheterization (OR = 3.1, 95% CI = 1.1-13.3), or nasogastric tube insertion (OR = 3.8, 95% CI = 1.8-8). These results show that some previously described risk factors for developing Enterobacter bacteremia were equally applicable in the case of Enterobacter gergoviae infections. In addition, the report represents an important contribution to establishing E. gergoviae as a relevant human pathogen with epidemiological potential, as it is the first case-control report in the region and one of a few in the world, analyzing outbreaks of bacteremias in the neonatal intensice care unit (ICU) caused by E. gergoviae.

Key words: neonatal intensive care unit, bacteremia, *Enterobacter gergoviae, Enterobacter aerogenes*, risk factors

Introduction

Enterobacter spp. was not considered a cause of nosocomial infections until 1970. (1,2) In the last decades, however, hospital-acquired *Enterobacter* bacteremias have been increasingly reported, especially in intensive care units (ICUs). (3-8) In addition to that, species of the genus *Enterobacter* show increasing resistance to antimicrobial drugs. (1-3,5,9-11)

Enterobacter bacteremia rates are around 1 per 1 000 admissions for university hospitals and tertiary health care institutions. Rates are usually two to three times higher in specialized units, such as oncology departments. (1,4) In contrast to general hospitals, studies of *Enterobacter* bacteremias occurring in pediatric hospitals, (12-14) and in neonatal ICUs (6,15,16) are rather scarce.

Bacteremias caused by species of the genus *Enterobacter* are 1.3 to 2.5 times more common in males, neonates and the elderly (1,12) and mortality asso-

ciated with *Enterobacter* bacteremias ranges between 20%-35% in all age groups. (12)

Risk factors for *Enterobacter* bacteremia are different in adults and children, especially neonates. For neonates, immunosuppression of any cause, early gestational age, low birth weight, as well as usage of invasive devices (central venous catheters, endotracheal tubes, urinary catheters) and antimicrobial drugs, were connected with a greater risk of *Enterobacter* bacteremias. (1,4,6,14,17,18) Antimicrobial resistance, especially towards beta-lactam antibiotics, (19) in addition to other risk factors, was associated with several outbreaks caused by species of the genus *Enterobacter* in neonatal ICUs. (6,16,20,21)

In this case- control study we evaluated the risk factors for bacteremias caused by a rare human pathogen *Enterobacter gergoviae,* and *Enterobacter aerogenes* among neonates in the intensive care unit under conditions of nosocomial outbreak.

Materials and Methods

The investigation was conducted from January 1st, 1995 until December 31st, 1996 at the Neonatology Unit of the Department of Obstetrics and Gynecology University Hospital Split and was approved by the Ethics Committee of the University Hospital Split.

A matching case – control method was implemented. A case was every neonate admitted to the ICU, from whose blood culture *Enterobacter* spp. was isolated and who had clinical signs of illness. The control group represented neonates admitted to the Unit at the same time, with sterile hemocultures and no clinical signs of blood infection. Controls were matched according to gestational age, birth weight and time of hospitalization (only case-control pairs that were hospitalized at the same time). Cases were compared with respective controls in relation to their exposure to potential risk factors for enterobacter bacteremia.

The following potential risk factors for developing *Enterobacter* bacteremias were analyzed: a) duration and characteristics of delivery (vaginal or cesarean section; umbilical cord damage; prolapse or wrapping of the cord around the neonates neck; meconial, milky or bloody amniotic fluid; Apgar score), b) characteristics of the neonate in terms of gestational age and birth weight (premature or full term delivery; first or second twin; birth weight ; mode of hospitalization - incubator, baby-term, or standard bed), c) implementation of invasive procedures (mechanical ventilation; umbilical catheterization; nasogastric tube insertion), d) drugs used, e) blood derivatives received (concentrated erythrocytes; exsanguino-transfusion; thrombocytes; immuno-globulins; plasma; human albumins), f) way of feeding (breast, bottle, parenteral), g) microbial flora isolated from ear, nose, throat, rectum swabs and hemocultures on admission and during hospitalization, and h) maternal characteristics . Maternal characteristics evaluated as risk factors included: mother's age, education, place of residence, number of gestations, abortions, deliveries and complications during pregnancy. A detailed questionnaire with specified epidemiological data was developed and completed for every case and control.

Bacteremia was defined as a laboratory confirmed bloodstream infection. All neonatal infections, with the exception of proven transplancental infections, were considered nosocomial. (22) Bacteremias in neonates were classified as: very early (infections diagnosed within the first 48 hours from admission, usually acquired from the mother during passage through the birth canal), early (bacteremias developed after delivery, between 2 to 7 days of hospitalization), late (between 8 to 30 days of hospitalization) and very late (after more than 30 days of hospitalization). (22)

The rate of bacteremias was expressed as a proportion of patients with bacteremia relative to the number of admissions to the department.

Blood for hemocultures was placed on a liquid media for aerobic and anaerobic bacteria, and incubated in an automatized machine for hemoculture Vital (bio Merieux, Marcy d'Etoile, France). Identification of *Enterobacter* species was based on biochemical characteristics using standard microbiological methods, and confirmed with API 20E (bio Merieux). Susceptibility to antimicrobial drugs was determined using the disk diffusion method.

Pearson's chi-square and contingency tables were used for the analysis of qualitative variables. Differences in frequencies of the events between the groups were evaluated at the significance level of alpha=0.05.

For evaluating the strength of the relationship between risk factors and illness, odds ratio was used. 95% confidence interval, (95% CI), was used to indicate the reliability of an odds-ratio estimate.

Results

During the observed period of two years, a total of 64 (42 male and 22 female) cases were included, with an average body mass of 2795 g (ranging from 1050 to 4750 g) (table 1). Average length of stay in the ICU was 33.5 days (from 9 to 91 days). The control group consisted of 64 (42 male and 22 female) neonates, with an average mass of 2863 g (ranging from 1250 to 4200 g), and average length of stay in the ICU of 22 days (from 5 to 64 days).

In 1995, 21 bacteremias were diagnosed, and in 1996, 43 bacteremias. The rate of bacteremias per 1 000 admissions was 10 in 1995 and 22 in 1996, with an average rate of 16 per 1 000 admissions. Bacteremias were more common in male neonates with an average ratio of 1.9 relative to female neonates.

From a total of 64 neonates with bacteremia, 6 (9.4%) died, with bacteremia caused by *E. gergoviae*, so mortality connected with that bacteria was 13.9% (table 1). In the control group, no neonates died.

The distribution of incidence of bacteremias by the time of bacteremia onset and by the causal *Enterobacter* species is shown in table 2. Bacteremias most commonly occurred between 7th to 30th day of hospitalization (56.3%).

Table 1. Neonatal *Enterobacter* bacteremias observed at the Department of Neonatology intensive care unit, University Hospital Split, during 1995 and 1996.

Year	Bacteremias (n)	Bacteremias (rate /1000 admissions)	Gender (M:F)	Hemoculture isolates		Mortality	
				E. aerogenes (n)	*E. gergoviae* (n)	(n)	(%)
1995.	21	10	4:1	12	9	0	0
1996.	43	22	1.4:1	0	43	6	13.9
Total / Average	64	16	1.9:1	12	52	6	9.4

Table 2. Distribution of bacteremias by the time of the onset and the causal *Enterobacter* species.

Bacteremia (time of onset)	*E. aerogenes*		*E. gergoviae*		Total	
	(n)	(%)	(n)	(%)	(n)	(%)
Very early (within 48 h)	0	0	3	5.8	3	4.7
Early (2 to 7 days)	5	41.7	19	36.5	24	37.5
Late (8 to 30 days)	7	58.3	29	55.8	36	56.2
Very late (after 30 days)	0	0	1	1.9	1	1.6
Total	12	100	52	100	64	100

Table 3. Neonates that developed *Enterobacter* bacteremia according to maternal characteristics.

Characteristic of mothers	n	%
Mothers age (years)		
Less than 24 years	21	32.8
25-29 years	11	17.2
30-34 years	18	28.1
35 and more years	12	18.8
unknown	2	3.1
Mothers education		
primary school or no education	33	51.6
secondary school	25	39.1
university	6	9.3
Mothers place of living		
Split-urban area	27	42.2
Province hinterland	37	57.8
Gestation		
first	19	29.7
second	19	29.7
third and more	26	40.6
Delivery		
first	23	35.9
second	21	32.8
third and more	20	31.3

Very early and very late bacteremias were observed only in three and one case, respectively.

In table 3 the frequency distributions and percentages of newborns that developed nosocomial *Enterobacter* bacteremias are shown, in relation to the different characteristics of their mothers. Occurrence of bacteremias in the neonates was inversely proportional to the education of their mothers.

Regarding place of residence, neonates from mothers that lived in areas far from urban Split showed an increased tendency of developing bacteremia in comparison to neonates of mothers that lived in the city of Split, although this tendency did not reach statistical significance.

Colonization or infection of neonates prior to the onset of bacteremia was associated with a 3.4 (95% CI=1.2-9.9) and 7.9 (95% CI=1.2-52.5) times increased risk of developing bacteremia, due to *Enterobacter* and non-*Enterobacter* microbial agents, respectively (table 4).

The spectrum and incidence of use of different antimicrobial drugs was also

Table 4. Microbial isolates from biological specimens of cases and controls before the onset of bacteremia.

Microbial isolates	Cases		Controls	
	n	%	n	%
*Enterobacter**	11	17.2	4	6.3
non-*Enterobacter**	18	28.1	3	4.7
no microbial isolates*	35	54.7	57	89.0

* cases vs. controls, $p<0.05$
enterobacter = species of genus *Enterobacter* (OR=3.4, 95%CI=1.2-9.9)
non-enterobacter = species of genus *Staphylococcus, Streptococcus, Escherichia, Acinetobacter, Proteus, Pseudomonas, Candida* (OR=7.9, 95%CI=1,2-52.5).

Table 5. Use of antimicrobial drugs in neonates before the onset of bacteremia.

Antimicrobial drugs	Cases		Controls	
	n	%	n	%
ampicillin	62	96.9	54	84.4
gentamicin	19	29.7	19	29.7
amikacin*	44	68.8	6	9.4
netilmycin	37	57.8	32	50
ceftazidime*	33	51.6	6	9.4
cefuroxime	11	17.2	7	10.9

*cases vs. controls, $p<0.05$
amikacin (OR=7.5, 95%CI=2.8.-19.9)
ceftazidime (OR=7.8, 95%CI=1.6-38.6)

Table 6. Application of invasive interventions during hospitalization in cases and controls.

Invasive interventions	Cases		Controls	
	n	%	n	%
no intervention	42	65.6	55	85.9
mechanical ventilation*	20	31.3	5	7.8
nasogastric tube insertion*	37	57.8	17	26.6
umbilical catheterization*	11	17.2	4	6.3

*cases vs. controls, $p<0,05$
mechanical ventilation (OR=4.7, 95%CI=1.6-13.5)
nasogastric tube insertion (OR=3.8, 95%CI=1.8-8)
umbilical catheterization (OR=3.1, 95%CI=1.1-13.3)

higher in the group of cases and was associated with an increased risk of developing bacteremia in the case of amikacin and ceftazidime (table 5).
Invasive interventions such as mechanical ventilation, nasogastric tube use and/or umbilical catheter insertion, were significantly more often applied in the group of cases and were associated with a higher chance of developing bacteremia (table 6).
Other potential risk factors for developing enterobacter bacteremias that were analyzed (duration and mode of delivery, umbilical cord related abnormalities, meconium stained, milky or bloody amniotic fluid, Apgar score, first or second twin, mode of hospitalizatio-on, administration of blood derivatives, type of feeding) showed no significant influence on the chances for developing bacteremias relative to the controls (data not shown).

Discussion

In this case-control study we describe epidemiological characteristics of *Enterobacter* bacteremia outbreaks in a neonatal ICU and analyze major risk factors for development of bacteremias.
The nosocomial outbreak is indicated by the occurrence of 64 neonatal bacteremias during 2 years, with a crude rate of 16 per 1000 admissions. (22)
Enterobacter species isolated from the cases were *E. aerogenes* (18,6%) and *E. gergoviae* (82%), which is rather atypical, since the most frequently described *Enterobacter* isolates have been *E. cloacae* and *E. aerogenes*, and less frequently, *E. agglomerans* and *E. sakazakii.* (1,2,5,21,22)

Over the last decade, *E. aerogenes* has been identified more often as a cause of bacteremia outbreaks in hospitals (3,7) as well as in ICUs (16,23). Nosocomial outbreaks due to multiresistant *E. aerogenes* are an emerging concern in ICUs. Infections caused by this organism are often not detected at an early stage and are both difficult to control and to treat.

In contrast to these, *E. gergoviae* is a relatively rare human pathogen. (4,24) It was only once described as a cause of bacteremias in a neonatal ICU in Asia. (25) *E. gergoviae* was first described in 1976 (26) and in 1980 was characterized in more detail as a new species of *Enterobacteriaceae*, found in clinical specimens and the environment. (27) Therefore, this report represents an important contribution in establishing *E. gergoviae* as a relevant human pathogen with epidemiological potential.

Our study also confirms the relevance of various established risk factors for developing *Enterobacter* bacteremias. (15,17,18,20,21,28) Among these, we especially recognized the relevance of colonization or infection of neonates prior to the onset of bacteremias, excessive use of certain antibiotics and application of invasive interventions. Our results also verify that length of stay in the neonatal ICU as a risk factor. The fact that the majority of bacteremias occurred after 7 days of hospitalization in the neonatal ICU indicate that the ICU served as a reservoir for colonization and infection of the neonates. (29) We also noticed that some maternal characteristics, such as education and place of living, might be associated with an increased tendency for neonates to develop bacteremias.

Although each of the mentioned risk factors can be independently justified, their separate analysis might not be appropriate because of the apparent interrelationship between them. For example, we believe that the initial risk might in part originate from the mother. Namely, a lower education and residence in remote areas of the province point to the poor socio-economic status of these mothers, which may pose a risk for the health of their babies. On the other hand, a tendency to and severity of illness in such babies is generally increased, which in turn is associated with more aggressive therapeutic and diagnostic procedures, thereby closing the chain of risk factors.

Conclusion

This is the first report in Croatia and the neighboring region, as well as one of a few in the world, describing an outbreak of bacteremias in a neonatal ICU caused by *E. gergoviae*.

We also showed that some previously described risk factors for developing *Enterobacter* bacteremia were equally applicable in the present case. Increasing survival of neonates with low birth weight and gestational age that require implementation of invasive methods and antimicrobial drugs, provide a favorable environment for the continuous presence of *Enterobacter spp.* as a cause of bacteremias in the neonatal ICU. Better understanding of epidemic characteristics and risk factors provide essential grounds for planning effective ways for infection prevention, the ever-increasing challenge.

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