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Bloodstream Infections due to *Enterobacteriaceae* Among Neonates in Poland – Molecular Analysis of the Isolates

AGNIESZKA CHMIELARCZYK, MONIKA POBIEGA*, JADWIGA WÓJKOWSKA-MACH,
DOROTA ROMANISZYN, PIOTR B. HECZKO and MAŁGORZATA BULANDA

Chair of Microbiology, Kraków, Poland

A b s t r a c t

Bloodstream infections (BSIs) are associated with a significantly increased risk of fatality. No report has been found about the molecular epidemiology of *Enterobacteriaceae* causing BSI in neonates in Poland. The aim of this work was to determine the antibiotic resistance profiles, virulence gene prevalence, the epidemiological and genetic relationships among the isolates from *Enterobacteriaceae* causing BSI in neonates with birth weight < 1501 g. Antimicrobial susceptibility testing was performed. PCR was performed to identify the presence of common beta-lactamase genes, virulence genes. PFGE and MLST were performed. The surveillance group contained 1,695 newborns. The incidence rate for BSIs was 5.9%, the fatality rate 15%. The most common species were *Escherichia coli* (n = 24) and *Klebsiella pneumoniae* (n = 16). CTX-M-15 was found in 6 *E. coli*, 8 *K. pneumoniae*, 1 *Enterobacter cloacae* strains. Among *E. coli fim*H (83.3%), *ibe*A (37.5%), *neu*C (20.8%) were the most frequent. PFGE demonstrated unique pulsotypes among *E. coli*. *E. coli* ST131 clone was found in 7 *E. coli* strains. PFGE of 16 *K. pneumoniae* strains showed 8 pulsotypes. Five isolates from one NICU belonged to one clone. MLST typing revealed 7 different ST with ST336 as the most prevalent. This study provides information about resistance, virulence and typing of *Enterobacteriaceae* strains causing BSI among neonates. *E. coli* and *Klebsiella* spp. isolated in this study have completely different epidemiology from each other.

K e y w o r d s: *Enterobacteriaceae*, *Escherichia coli* ST131, *Klebsiella pneumoniae*, bloodstream infections, MLST, very-low birth weight neonates

Introduction

Bloodstream infections (BSIs) are the most common nosocomial infections among neonates, associated with a significantly increased risk of fatality (Stoll *et al.*, 2002). Newborns, especially those with very low birth weight (VLBW) are primarily at risk of developing late-onset BSIs (LO-BSIs), caused by organisms acquired perinatally or postnatally, usually as a consequence of nosocomial transmission. The main risk factors for LO-BSI include prematurity, prolonged stay in a neonatal intensive care unit (NICU) and administration of invasive procedures (Ozkan *et al.*, 2014). Consequently, the improved survival rates of small premature infants experiencing long stays in modern NICUs, which are better equipped for life-saving intensive care, has been a major factor in the increase in LO-BSI caused by Gram-negative bacteria (Cordero *et al.*, 2004; Wojkowska-Mach *et al.*, 2013). *Enterobacteriaceae* are second only to coagulase-negative staphylococci (CoNS) in causing LO-BSIs (Karlowicz *et al.*, 2000; Lutsar *et al.*, 2014). Prolonged use of broad-spectrum antibiotics in NICUs is associated with the occur-

rence of multi-drug resistant (MDR) bacteria. Selection of more broadly resistant Gram-negative enteric bacteria is linked with outbreaks of bacterial disease in NICUs (Cordero *et al.*, 2004).

Nosocomial isolates such as *Escherichia coli* and *Klebsiella* sp. often have extended spectrum β-lactamase (ESBL) phenotypes, and *E. coli* strains carrying these enzymes have disseminated into the community. One of the best examples of this trend is the global spread of the clonal *E. coli* sequence type (ST) 131 (ST131), which expresses CTX-M enzymes. (Birgy *et al.*, 2013) In contrast, among *Klebsiella* strains, relatively few of the serotypes (particularly K1 and K2) appear to be linked with invasive strains. Also, the hypermucoviscous phenotype, strongly associated with the presence of *rpm*A and *mag*A genes, is a virulence marker in clinical strains (Jung *et al.*, 2013). In *Klebsiella pneumoniae*, CTX-M-15 is mainly associated with quinolone-resistant strains, belonging to ST11 clone (Damjanova *et al.*, 2008; Oteo *et al.*, 2009).

Despite many reports of ESBL-producing isolates among Polish hospitals, no report has been found about the molecular epidemiology of *Enterobacteriaceae*

* Corresponding author: M. Pobiega, Chair of Microbiology, Krakow, Poland; e-mail: monika.pobiega@gmail.com

causing BSI in neonates. Here, we collected clinical isolates from *Enterobacteriaceae* family bacteria originating from BSIs of neonates hospitalized in six Polish NICUs to determine their antibiotic resistance profiles, virulence gene prevalence, and the epidemiological and genetic relationships among the isolates. Here we have focused on detailed molecular studies, data about epidemiology of bloodstream infections in newborns are included in other manuscripts (Wojkowska-Mach *et al.*, 2014).

Experimental

Materials and Methods

Study population. Prior to the study, a confirmatory ethics vote for the data collected in the Polish Neonatology Surveillance Network (PNSN) for the scientific purpose was approved by the Bioethics Committee of Jagiellonian University Medical College (No KBET/221/B/2011).

Continuous prospective target surveillance of infections was conducted from 1/1/2009 through 12/31/2011 at Polish NICUs (only teaching hospitals) which participated in PNSN. These tertiary NICUs provided care for 20% of all VLBW infants born in Poland annually. The surveillance included infants hospitalized in these NICUs whose birth weights were < 1500 grams (VLBW) at birth until they achieved a weight of 1800 grams or died. The study covered 1,695 newborns. The general fatality case rate was 16.3%. All VLBW infants of suspected or documented infected were subject to registration regardless of the time of occurrence according to criteria of Gastmeier *et al.* (2004) when they had clinical signs of a bloodstream infection (BSI) (Gastmeier *et al.*, 2004).

- at least 2 of the following: temperature > 38°C or < 36.5°C or temperature instability, tachycardia or bradycardia, apnea, prolonged capillary refill, metabolic acidosis, hypoglycemia and other signs of bloodstream infections such as lethargy;
- and patients who had 1 of the following criteria: C-reactive protein > 2.0 mg/dl, immature/total neutrophil ratio (I/T ratio) > 0.2, leukocytes < 5000/µl and platelets < 10000/µl.

LO-BSI was defined when diagnosed > 72 hours after delivery. Clinical sepsis represented an infant where signs of infection existed but on blood culture a causative organism was not identified.

Central venous catheter (CVC)-associated BSI (CVC-BSI) and peripheral intravenous catheter (PVC)-associated BSI (PVC-BSI) were defined as infections associated with the use of a central or peripheral venous catheter within the preceding 48 hours prior to the onset of the infection (Gastmeier *et al.*, 2004).

Bacterial strains. The present study examined 55 *Enterobacteriaceae* family isolates originating from BSIs: 4 from early onset infections (EOI), 51 from LOI. The majority originated from NICUs VI and II (25 and 12, respectively). The remainder were from V (8), I (5), III (3) and IV (2).

Culture and species identification. All blood specimens of at least 1 ml were injected into an aerobic blood culture bottle (Bactec Plus 26 Aerobic; Becton Dickinson, Poland), and cultured on MacConkey agar, Columbia agar (at 37°C, each for 24 h) and Sabouraud agar (at 37°C, 48 hours) (all from BIOCORP, Poland). Collection and identification of the bacterial strains was performed in the local microbiology laboratories of each hospital. Isolates were identified by the automated identification system (VITEK 2; bioMérieux, Warsaw, Poland). Bacterial strains were stored at −70°C and sent to Department of Microbiology, Krakow.

Antimicrobial susceptibility tests. Isolates were tested using disk diffusion antimicrobial susceptibility methods on Mueller-Hinton agar plates according to the current European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using clinical breakpoint tables v. 3.1 (http://www.eucast. org v.3.1, accessed: 11.02.2013). All discs were obtained from Oxoid, Basingstoke. ESBL activity was detected using a modified double-disk synergy test (Drieux *et al.*, 2008). ESBL-positive strains resistant to at least two other groups of antibiotics were considered to be MDR.

DNA isolation. DNA templates were extracted using a Genomic Mini kit (A&A Biotechnology, Poland) according to manufacturer's instructions.

Polymerase chain reaction (PCR) for extended-spectrum β-lactamase genes and virulence factor screening. PCR was performed to identify the presence of bla_{CTX-M}, bla_{SHV} and bla_{TEM} genes (Chmielarczyk *et al.*, 2013; Monstein *et al.*, 2007), products were sequenced by commercial company (Genomed, Warsaw, Poland).

E. coli was checked for the presence of selected virulence genes as described elsewhere (Chmielarczyk *et al.*, 2013; Johnson and Stell, 2000; Watt *et al.*, 2003).

The isolates were classified to 1 of the 4 main *E. coli* phylogenetic groups (A, B1, B2, and D) (Chmielarczyk *et al.*, 2013; Clermont *et al.*, 2000).

K. pneumoniae was tested for the presence of *rmp*A (regulator of mucoid phenotype A), *mag*A (mucoviscosity-associated gene A), *wab*G (involved in the biosynthesis of the outer core lipopolysaccharide), *uge* (encoding uridine diphosphate galacturonate 4-epimerase, which is responsible for capsule biosynthesis), *kfu* (iron uptake system), and *all*S (encoding the activator of the allantoin regulon) (Brisse *et al.*, 2009). PCR was performed with primer pairs specific for the *K. pneumoniae* capsule gene cluster (*cps*K1, *cps*K2)

(Fang *et al.*, 2007; Turton *et al.*, 2008). NCTC 5054 and NCTC 5055 were used as positive controls for *cps*K1 and *cps*K2, respectively.

Pulsed-field gel electrophoresis (PFGE). All isolates were analyzed using the standardized PFGE protocol developed at the Centers for Disease Control and Prevention by the PulseNet program. *Xba*I (Thermo Scientific) was used for DNA digestion. The digested products were separated on a CHEF III PFGE system (BioRad, Warsaw, Poland) in 0.5× Tris-borate-EDTA buffer at 14°C at 6 V for 20 h with a ramped pulse time of 2.2–54.2 s for *E. coli* and 14°C at 6 V for 22 h with a ramped pulse time of 2–35 s for *K. pneumoniae, Klebsiella oxytoca, Enterobacter cloacae* and *Serratia marcescens.* GelCompar (Applied Maths, Kortrijk, Belgium) was used for cluster analysis with the unweighted pair group method with an arithmetic mean and the Dice coefficient similarity require to be > 90% for the pattern to be considered as belonging to the same type.

Multilocus sequence typing (MLST). MLST for *E. coli* was performed in accordance with (Wirth *et al.*, 2006) (http://mlst.ucc.ie/mlst/dbs/Ecoli), for *K. pneumoniae* was performed in accordance with (Diancourt *et al.*, 2005) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

Results

The surveillance group contained 1,695 newborns with birth weight below 1500 g. Data on gestational age, mode of delivery, birth weight, multiple birth were previously published (Wojkowska-Mach *et al.*, 2014). In the study group, 100 cases of *Enterobacteriaceae* BSI were registered. Among all the etiological factors for BSI, bacteria belonging to Enterobacteriaceae family were responsible for 15.2% of such infections. The incidence rate for Enterobacteriaceae BSIs was 5.9%, while the fatality rate was 15%. The fatality rate for *E. coli* was 26.0% and for *Klebsiella* sp. 10.0% (p = 0.09). Of the 55 *Enterobacteriaceae* isolates included in our study, there were 24 *E. coli*, 16 *K. pneumoniae*, 4 *K. oxytoca*, 5 *E. cloacae*, 1 *Enterobacter sakazakii*, 4 *S. marcescens* and 1 *Morganella morganii* (Table I).

The CVC utilization rate was 0.45 and the PVC utilization rate was 0.16 (calculated by dividing the number of days of CVC/PVC by the total number of patient days). Among the infections caused by *E. coli*, 20.8% of LO-BSIs were associated with CVC use, while 8.3% were associated with PVC use. Among the LO infections caused by *K. pneumoniae*, 43.75% were associated with CVC and 18.75% with PVC.

Table I
Participation of particular isolates in species and NICU.

NICU	*Escherichia coli* (n = 24)			*Klebsiella pneumoniae* (n = 16)			*Entero-bacter* sp. (n = 6)	*Klebsiella oxytoca* (n = 4)	*Serratia marcescens* (n = 4)	*Morganella morganii* (n = 1)
		ST	*Pulsotype*		*ST*	*Pulsotype*				
I (n = 5)	1	ST141	unique	1	ST153	unique	2			
	1	ST73	unique							
II (n = 12)	4	ST131	unique				1	3		
	2	ST69	identical (the same patient)							
	1	ST69	unique							
	1	ST75	unique							
III (n = 3)				1	ST336	unique			1	1
IV (n = 2)									2	
V (n = 8)	2	ST131	unique	2	ST17	identical (the same patient)		1		
	1	ST141	unique							
	1	ST69	unique							
	1	ST543	unique							
VI (n = 25)	3	ST998	unique	5	ST336	clone A	3		1	
	1	ST131	unique	2	ST11	clone B				
	1	ST141	unique	2	ST6	clone C				
	1	ST69	unique	1	ST6	unique				
	1	ST95	unique	1	ST153	unique				
	1	ST569	unique	1	ST870	unique				
	1	non typeable	unique							

Table II
Non-susceptible strains among different species of *Enterobacteriaceae* family.

	Escherichia coli n = 24 (%)	*Klebsiella pneumoniae* n = 16 (%)	*Enterobacter* sp. n = 6 (%)	*Klebsiella oxytoca* n = 4 (%)	*Serratia marcescens* n = 4 (%)	*Morganella morganii* n = 1 (%)
Penicillins						
Ampicillin	22 (91.7)	16 (100)	nd	4 (100)	nd	nd
Amoxicillin-clavulanate	13 (54.2)	9 (56.3)	nd	2 (50)	nd	nd
Piperacillin-tazobactam	0 (0)	2 (12.5)	1 (16.7)	0 (0)	0 (0)	0 (0)
Cephalosporins						
Cefepime	6 (25)	9 (56.3)	0 (0)	0 (0)	0 (0)	0 (0)
Cefotaxime	5 (20.8)	9 (56.3)	2 (33.3)	0 (0)	1 (25)	1 (100)
Ceftazidime	6 (25)	9 (56.3)	1 (16.7)	0 (0)	1 (25)	0 (0)
Cefuroxime	6 (25)	9 (56.3)	nd	0 (0)	nd	nd
Carbapenems						
Doripenem	1 (4.2)	3 (18.8)	0 (0)	0 (0)	0 (0)	0 (0)
Ertapenem	2 (8.3)	3 (18.8)	2 (33.3)	0 (0)	0 (0)	1 (100)
Imipenem	1 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Meropenem	1 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Monobactams						
Aztreonam	8 (33.3)	13 (81.3)	2 (33.3)	1 (25)	1 (25)	1 (100)
Fluoroquinolones						
Ciprofloxacin	5 (20.8)	7 (43.8)	0 (0)	0 (0)	0 (0)	0 (0)
Aminoglycosides						
Amikacin	2 (8.3)	2 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)
Gentamicin	3 (12.5)	3 (18.8)	0 (0)	0 (0)	0 (0)	0 (0)
Tobramycin	6 (25)	8 (50)	0 (0)	0 (0)	0 (0)	1 (100)
Tetracyclines						
Tigecycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Miscellaneous						
Chloramphenicol	1 (4.2)	3 (18.8)	0 (0)	0 (0)	0 (0)	0 (0)
Trimethoprim-sulfamethoxazole	10 (41.7)	6 (37.5)	0 (0)	0 (0)	0 (0)	0 (0)

The highest level of resistance among *E. coli* isolates was observed against AMP (91.7%), AMC (54.2%), SXT (41.7%) and ATM (33.3%). The highest level of resistance among *K. pneumoniae* isolates was observed against AMP (100%), ATM (81.3%), AMC (56.3%), all the cephalosporins investigated herein (56.3%), and CIP (43.8%) (Table II). The ESBL phenotype was found among 16 isolates (29%), with nine *K. pneumoniae*, six *E. coli* and one *E. cloacae*.

Molecular characterization showed that all ESBL-positive isolates carried the bla_{CTX-M} gene: 15 of them had CTX-M-15 and one had CTX-M-3. Eleven isolates (68.8%) harbored the TEM-1 gene together with the CTX-M gene. Five isolates harbored the SHV-11 gene and one harbored SHV-1 together with CTX-M. Four isolates harbored CTX-M-15 gene together with TEM-1 gene and SHV-11 gene. One isolates harbored CTX-M-15 gene together with TEM-1 gene and SHV-1 gene. Ten of the ESBL-positive strains (5 *E. coli* and 5 *K. pneumoniae*) were regarded as MDR types.

The most frequently detected genes among the *E. coli* isolates were iron-related genes including *fhu*A (91.7%), *fec*A (75%), *iut*A (58.3%), *fyu*A (54.2%), *iro*N (50%) and *iuc*C (50%), *iha* (8.3%). The most frequently detected adhesion gene was *fim*H (found in 83.3% of the isolates). The *ibe*A gene was found in 37.5% of isolates, while the *neu*C gene was found in 20.8% of isolates.

Sixteen (66.6%) isolates clustered in the ECOR group B2, 6 (25.0%) in D, 1 (4.2%) in A, and 1 (4.2%) in group B1.

None of the *K. pneumoniae* strains tested here were the capsular K1 or K2 type. Additionally, the *mag*A gene was not identified in the isolates. In contrast, the gene *uge* was detected in all of the *K. pneumoniae* strains, while *wab*G was present in 11 of them (68.8%). Only one isolate harbored *all*S (no 75), while another harbored *kfu* and *rpm* genes (no 220).

PFGE typing demonstrated that almost all of the 24 *E. coli* isolates had unique pulsotypes. Two strains with identical pulsotypes were isolated from the same patient.
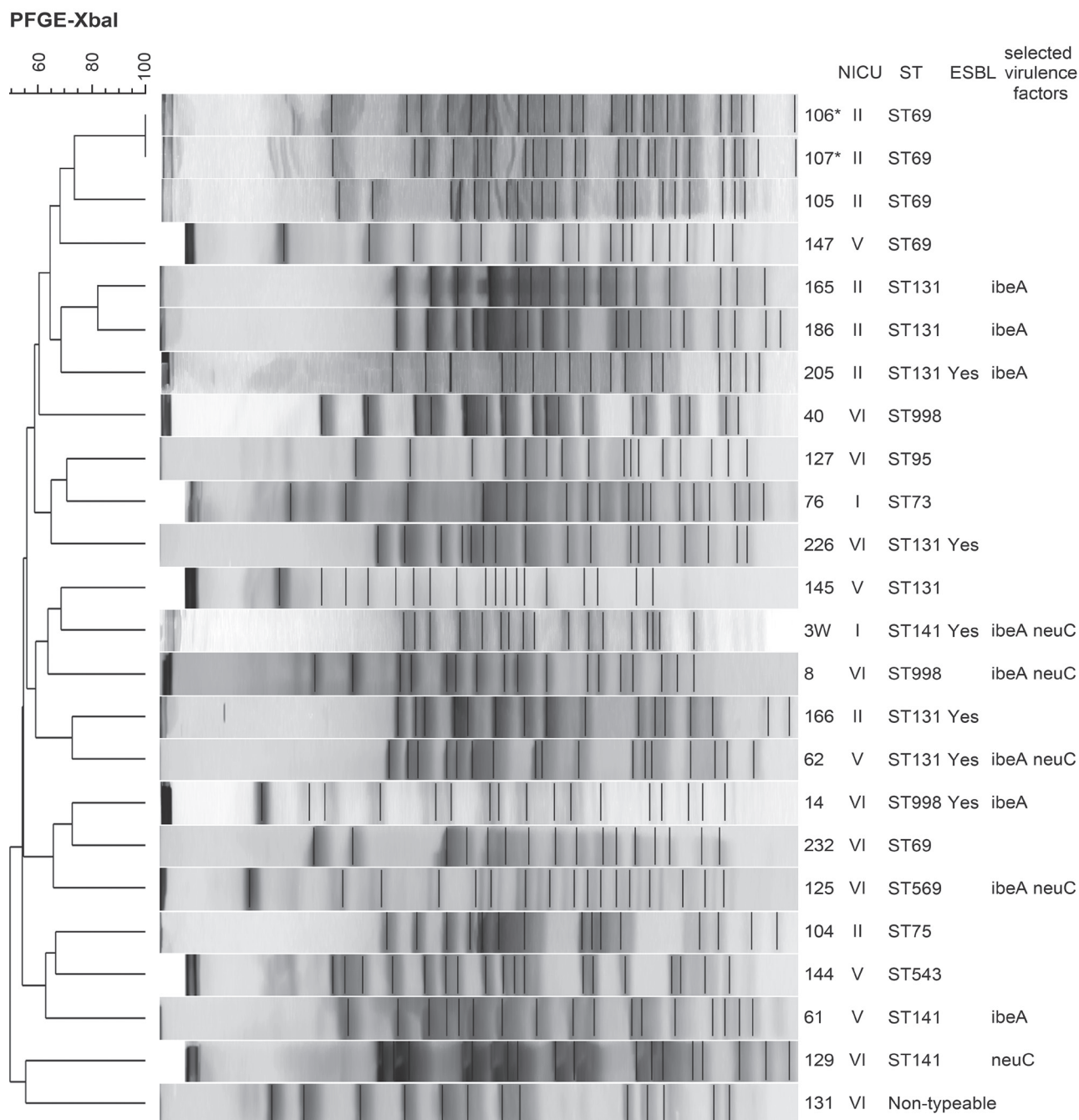
PFGE-XbaI

Fig. 1. Dendrogram of *E. coli* isolates. NICU was designated by Roman numerals. Asterisks indicate isolates derived from the same patient.

MLST typing revealed 10 different sequence types; these included ST131 (7 isolates), ST69 (5), ST141 (3), ST998 (3), ST73 (1), ST75 (1), ST95 (1), ST543 (1) and ST569 (1). One isolate could not be typed. Four among the ST131 isolates were from NICU II (Fig. 1). PFGE typing of 16 *K. pneumoniae* strains showed eight different pulsotypes. Five isolates from NICU VI belonged to one clone A. Those isolates were detected in 2009, 2010 and 2011. The mortality connected with those strains was 21.4%. Clone B consisted of two isolates similarly to clone C while the remaining five isolates had unique pulsotypes. MLST typing revealed seven different sequence types: ST 336 (6 isolates), ST6 (3), ST 11 (2) ST17 (2), ST153 (1), ST321 (1), and ST 870 (1). The main clone identified by PFGE was compatible with ST 336 (Fig. 2). PFGE typing of the *E. cloacae* strains revealed unique pulsotypes. Among *K. oxytoca*, two strains with identical pulsotypes were from the same patient, as was in the case for *S. marcescens* (data not shown).

Discussion

Enterobacteriaceae remain one of the most important, albeit not the most frequent, cause of BSIs. Septicaemia or BSI in neonates is an important cause of neonatal mortality and morbidity in developing
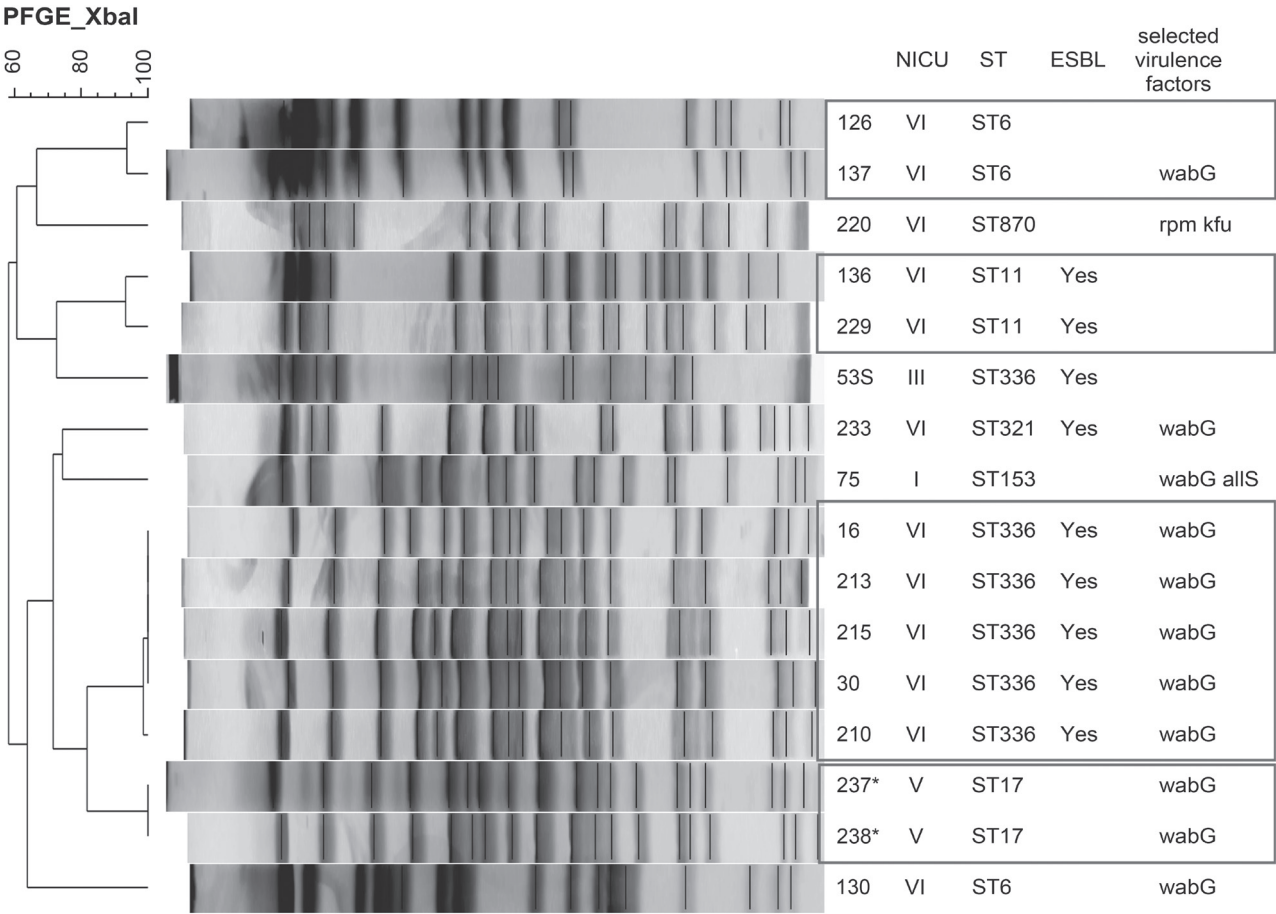
PFGE_XbaI

	NICU	ST	ESBL	selected virulence factors
126	VI	ST6		
137	VI	ST6		wabG
220	VI	ST870		rpm kfu
136	VI	ST11	Yes	
229	VI	ST11	Yes	
53S	III	ST336	Yes	
233	VI	ST321	Yes	wabG
75	I	ST153		wabG allS
16	VI	ST336	Yes	wabG
213	VI	ST336	Yes	wabG
215	VI	ST336	Yes	wabG
30	VI	ST336	Yes	wabG
210	VI	ST336	Yes	wabG
237*	V	ST17		wabG
238*	V	ST17		wabG
130	VI	ST6		wabG

Fig. 2. Dendrogram of *K. pneumoniae* isolates. NICU was designated by Roman numerals. Detected clones were marked by frames. Asterisks indicate isolates derived from the same patient.

countries and *K. pneumoniae* and *E. coli* are prominent causative agents. In a study of 6215 infants admitted to the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) centers, 70% of first episode late-onset infections were caused by Gram-positive organisms, with coagulase-negative staphylococci accounting for 48% of the infections; however, the death rates were highest for infants infected with *E. coli. (*Karlowicz *et al.*, 2000*)* Studies conducted by Makhoul *et al.* (2005) showed that mortality rate was about 4 times higher for infections caused by *E. coli* and 6 times higher for *K. pneumoniae* than for CoNS infections. Gram-negative bacilli (GNB) have been shown to be the non-dominant group of etiological risk factors for BSI, representing 20–30% of the infections in some studies (Graham *et al.*, 2006; Mitt *et al.*, 2014; Nagao, 2013). A study conducted by Cordero *et al.* (2004) showed that 20% of BSI episodes were caused by GNB, of which 15% were *Enterobacteriaceae*. Identification of these microorganisms in our study group was 15.2% and, importantly, infection with these etiological agents has been shown to be connected with high mortality rates in neonates (15%). Stoll *et al.* (2011) has described *E. coli* as the major causative patho-

gen of BSIs in preterm infants and the second most common cause of BSIs in term infants. Vergnano *et al.* (2011) indicated that *E. coli* was the third most common microorganism isolated from neonates and reported a prevalence of 13% in the UK, whereas in Germany the prevalence was 4.8% (Geffers *et al.*, 2008), which is about three times less than that observed in NICUs in the present study. Unfortunately, *E. coli* is frequently associated with severe infections and is the leading cause of sepsis-related mortality among VLBW infants. Indeed, *E. coli* accounted for 24.5% of EOI of neonates in the United States (Weston *et al.*, 2011) and 14.6% in Polish VLBW infants (Chmielarczyk *et al.*, 2014). In the present study, other Enterobacteriaceae family microorganisms such as *Klebsiella* spp. and *Enterobacter* spp. were markedly less common, similar to other studies (Cordero *et al.*, 2004; Geffers *et al.*, 2008; Lombardi *et al.*, 2014; Nagao, 2013; Vergnano *et al.*, 2011).

Crossing the skin barrier provides a direct route of invasion for bacteria; therefore, insertion of a CVC and the length of its duration *in situ* are risk factors for the development of a BSI. Lombardi *et al.* (2014) showed that in the neonatal pathology ward CVC infections reached 36.3% among Gram-negative bacteria, and

Klebsiella spp. were the most frequently isolated. This result is similar to our data.

E. coli and *Klebsiella* spp. isolated from NICUs in this study have different epidemiology to each other; hence, they require different surveillance and infection control. According to PFGE results, *E. coli* comprised different clones, while among the *K. pneumoniae* isolates, some showed high genetic similarity. Similar findings have been reported previously by other authors. (Castro *et al.*, 2010; Wojkowska-Mach *et al.*, 2013) Of the six NICUs we investigated, the majority of the *Enterobacteriaceae* isolates were from NICU VI, where *Klebsiella* dominated; this finding may be related to clonal spread of these bacteria. The *Klebsiella* clones detected in our study belong to four sequence types (ST): ST6, ST11, ST17 and ST336, of which ST11 and ST17 are known pathogens and colonizers widely distributed in Europe (Damjanova *et al.*, 2008; Oteo *et al.*, 2009).

The MLST technique showed that seven *E. coli* strains belonged to ST131, but according to the PFGE results, these strains had unique pulsotypes and came from three different centers; hence, they are assumed not to be clonally spread. It has been reported that strains belonging to the same ST do not always cluster in a single branch based on cluster analysis of PFGE patterns (Vimont *et al.*, 2008). ST131 is often reported in adults and recently also in children and neonates, and is probably transmitted between mothers and neonates (Denkel *et al.*, 2014). The spread of ESBL-producing ST131 *E. coli* in the community, especially during the neonatal period, is a cause for serious concern (Birgy *et al.*, 2013). Recently, a CTX-M-15 ESBL-positive *E. coli* ST131 clone, belonging to the B2 phylogenetic group and characterised by a particular antimicrobial resistance and high virulence potential, became a major public health problem especially in developing countries (Brisse *et al.*, 2012; Clermont *et al.*, 2009).

The microorganisms responsible for neonatal BSI have changed over time and vary from place to place. Prolonged use of broad-spectrum antibiotics in NICUs is associated with the current epidemic of MDR Gram-negative bacteria. Biedenbach *et al.* (2004) reported that the neonatal sepsis rate from *Klebsiella* spp. was higher in Latin America than in North America and was correlated with the use of extended-spectrum antibiotics. *Klebsiella* spp. and *E. coli* had the highest level of resistance to ampicillin, amoxicillin and aztreonam, which may have been caused by frequent administration of these drugs. Studies about antibiotic consumption conducted on the Polish NICUs have shown that beta-lactams were administred in more than 50% of infections (Rozanska *et al.*, 2012). In our study, higher levels of antibiotic resistance (and more ESBL-positive strains) were observed among *Klebsiella* strains, probably as a result of the epidemic clone detected in NICU VI.

The *E. coli* isolates studied here were more likely to posses the *ibe*A gene than those derived from urinary and respiratory tract infections (Chmielarczyk *et al.*, 2013). Soto *et al.* found that strains causing EOI harbored a higher frequency of the *ibe*A gene (Soto *et al.*, 2008). Among *Klebsiella* sp. only some of the isolates possessed virulence genes such as *rpm*A or *kfu*. None of the isolates possessed K1 or K2 serotypes. As the capsule is a major virulence factor for *K. pneumoniae* (Cortes *et al.*, 2002), information about the prevalence of the capsular types in such infections is an essential component of infection prevention and control (Pan *et al.*, 2013).

According to the results, contact isolation is required when a patient is colonized or infected by a microorganism which is transmitted by direct physical contact. The presented results have shown that reliable adherence to that principle by the personnel is necessary when *Klebsiella* spp. is highly level of endemic or in case of epidemic. However, data about *E. coli* showed that precautions should be different and regimen about hand hygiene is insufficient. In the case of *E. coli* surveillance on people who take care of a neonate including mother and people performing Kangaroo care, could be performed. Infection prevention should also require intensive education and preparing procedures about Kangaroo care, feeding and milk expression (including milk storage) (Guzman-Cottrill *et al.*, 2013).

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