

SPECIES COMPOSITION AND RESISTANCE TO ANTIMICROBIAL AGENTS OF *KLEBSIELLA* SPP. STRAINS ISOLATED FROM FAECES AND BLOOD CULTURES OF NEONATES WITH COLONIZATION AND INFECTION

K. Dragoev, M. Sredkova, V. Grigorova, V. Edreva, V. Popova,
H. Hitkova, I. Ivanov

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Abstract

A total of 118 strains of *Klebsiella* spp. were analysed, of them 114 (96.61%) were isolated from faecal samples of neonates with colonization of the digestive tract, and 4 (3.39%) from blood cultures of neonates with sepsis. Isolated strains were identified to belong to two species as follows: *Klebsiella pneumoniae*: a total of 114 (96.61%) strains, of them 110 (93.22%) strains from faeces and 4 (3.39%) from blood cultures, and *Klebsiella oxytoca* – 4 (3.39%) from faeces. Production of extended spectrum β -lactamases (ESBLs) was recorded in 73 (61.86%) and 4 (3.39%) strains of *Klebsiella pneumoniae* isolated from faeces and blood cultures, respectively, as well as in two (1.69%) strains of *Klebsiella oxytoca* isolated from faeces. Strains of *Klebsiella pneumoniae* isolated from faeces displayed high incidence of resistance to ampicillin, gentamicin and cefamandole (94.55, 89.09 and 87.27%, respectively), and to cefotaxime, ceftriaxone and ceftazidime (83.64, 81.82 and 81.82%, respectively), as well as low resistance to piperacillin/tazobactam and amikacin (5.45 and 13.64%, respectively). The characteristics of ESBLs in 4 strains of *Klebsiella pneumoniae* isolated from blood cultures of neonates with sepsis determine their high incidence of resistance to ampicillin, azlocillin, piperacillin, as well as to cephalosporins of 2nd and 3rd generation, but at the same time they manifested high susceptibility to cefoxitin, amoxicillin/clavulanic acid, piperacillin/tazobactam, amikacin and ciprofloxacin. The low values of resistance of *Klebsiella* spp. strains to piperacillin/tazobactam and amikacin allowed application of both medicaments as means of choice for empirical treatment of newborn babies with infection and colonization caused by *Klebsiella* spp.

Key words: *Klebsiella* spp., *K. pneumoniae*, *K. oxytoca*, resistance to antibacterial agents, extended spectrum β -lactamases (ESBLs), colonization and infection

Klebsiella spp. are opportunistic microorganisms causing severe diseases, such as septicaemia, pneumonia, infections in the urinary tract, infections in the soft tissues, etc. *Klebsiella* spp. are among the most important nosocomial pathogens causing hospital infections. From 5 to 7% of the nosocomial infections are due to these microorganisms [1]. Multiple-resistant strains of *K. pneumoniae* are a serious problem in a great number of hospitals throughout the world [2]. *Klebsiella*-induced infections are widespread in neonatological intensive care units (NICUs) leading to septicaemia and

meningitis in new-born children. These infections are caused by “neonatal” clones representing a serious problem for neonates with nosocomial infection in NICUs. Most frequently the hospital outbreaks of infections are caused by multiple-resistant *Klebsiella* spp. strains, producers of extended spectrum β -lactamases (ESBLs). ESBLs strains are now a world-wide problem for hospitalized patients [3]. *K. pneumoniae* strains producing ESBLs (EPKP) are often involved in the outbreaks of hospital infections spread in NICUs [4].

Neonates are devoid of endogenic microflora and owing to this they are susceptible to colonization of Gram-negative microorganisms from the environment during their stay in NICU. There are two major pathways in neonates to acquire pathogenic microflora: by perinatal transfer from maternal vaginal flora (vertical transfer) and after birth from environment or from sources of human origin (horizontal transfer). Acquired postnatal microflora from environment in NICU increases in cases of premature labours, as well as in cases of neonates staying for a long time in the hospital [5].

Frequent administration of antibiotics in NICU is a high-risk practice leading to colonization of new-born babies with nosocomial strains of multiple-resistant Gram-negative bacteria [5].

The aim of this study was to follow up at regional level of the Institute Hospital in Pleven, Bulgaria, the species composition and resistance to antimicrobial agents of *Klebsiella* spp. isolated from faeces of neonates with colonization of the digestive tract, as well as with sepsis.

Material and methods. A total of 118 strains of *Klebsiella* spp. were subjected to analysis, of them 114 strains were isolated from faeces of new-born infants with colonization of the digestive tract, and 4 strains from blood cultures of neonates with sepsis at the Neonatological Clinic in the Institute Hospital in Pleven within a period of 18 months (August 1, 2001 – January 31, 2003).

All faecal samples were cultured on Levine medium and apocholate-citrate agar. Blood samples were collected using sterile implements and then cultured in flasks containing Bactec Peds Plus/F medium. The blood cultures were incubated in Bactec 9120 apparatus (Becton Dickinson). They were used to make Gram-stained preparations, as well as subcultures on chocolate blood agar (in 8–10% CO₂), blood agar (with 5% sheep blood) and Levine medium.

Strains isolated from faecal samples were biochemically identified by routine methods, and their susceptibility was determined by the method of Bauer-Kirby [6].

Identification and susceptibility to antibiotics of the strains from blood cultures were analysed with the aid of miniAPI system (bioMerieux).

Production of ESBLs was determined by the double-disc synergic test (DDST) known also as disc-diffusion screening test including the following antibacterial agents: ceftazidime (30 μ g), ceftriaxone (30 μ g), cefotaxime (30 μ g) and amoxicillin/clavulanic acid (20/10 μ g per disc). The results were interpreted in accordance with the criteria published by the National Committee for Clinical Laboratory Standards (NCCLS) 1999 in the USA [7].

Results and discussion. Table 1 shows the species composition of 118 strains of *Klebsiella* spp. isolated from faeces of neonates with colonization of the digestive tract and with sepsis. The isolated strains were identified as belonging to two species, as follows: *K. pneumoniae* 110 (93.22%) strains from faeces and 4 (3.39%) strains from blood cultures, and *K. oxytoca* 4 (3.39%) strains from faeces.

Our results demonstrated that nosocomial *Klebsiella* infections (colonization and sepsis) in neonates are caused mainly by *Klebsiella pneumoniae*, from medical point of view the most important species of genus *Klebsiella*, and to a lower extent by *K. oxytoca*, a standpoint which coincides with the conclusions of the German authors R. PODSCHUN and U. ULLMANN [1] who considered *Klebsiella* spp. as nosocomial pathogens.

Table 1

Species and resistance to antimicrobial agents of 118 strains of *Klebsiella* spp. isolated from faeces and blood culture of new-borns with colonization in the digestive tract and with sepsis-infection

Antimicrobial agents	No (%) of resistant <i>Klebsiella</i> strains								
	Faeces/colonization						Blood (infection)		
	KP	EPKP	Total $n = 110$		KO	EPKO	Total	KP	EPKP
	$n = 37$	$n = 73$	R	S	$n = 2$	$n = 2$	$n = 4$	$n = 0$	$n = 4$
Ampicillin	34 (91.89)	70 (95.89)	104 (94.55)	6 (5.45)	2	2	4		4 (100)
Azlocillin								4 (100)	4 (100)
Piperacillin								4 (100)	4 (100)
Cefuroxime								4 (100)	4 (100)
Cefamandole	23 (62.16)	73 (100)	96 (87.27)	14 (12.73)	0	2	2 (100)	4 (100)	4 (100)
Cefoxitin	5 (13.51)	15 (20.54)	20 (18.18)	90 (81.82)	0	2	2	0	0
Cefotaxime	19 (51.35)	73 (100)	92 (83.64)	18 (16.36)	0	2	2	4 (100)	4 (100)
Ceftriaxone	17 (45.94)	73 (100)	90 (81.82)	20 (18.18)	0	2	2	4 (100)	4 (100)
Ceftazidime	17 (45.94)	73 (100)	90 (81.82)	20 (18.18)	0	2	2	4 (100)	4 (100)
Amoxicillin/ Clavulanic acid	10 (27.02)	51 (69.86)	61 (55.45)	49 (44.55)	0	1 1	1 1	1 0	1 0
Piperacillin/ Tazobactam	0	6 (8.21)	6 (5.45)	104 (94.55)	0	2	2	0	0
Gentamicin	27 (72.97)	71 (97.26)	98 (89.09)	12 (10.91)	0	2	2	4 (100)	4 (100)
Amikacin	4 (10.81)	11 (15.06)	15 (13.64)	95 (86.36)	0	0	0	0	0
Ciprofloxacin								0	0

KP – *K. pneumoniae*; EPKP – *K. pneumoniae* producer of extended spectrum β -lactamases;
 KO – *K. oxytoca*; EPKO – *K. oxytoca* producer of extended spectrum β -lactamases

Of all *Klebsiella* strains analysed here, production of ESBLs was found in 79 (66.94%) strains: of them EPKP were 73 (61.86%) strains isolated from faeces of neonates with colonization of the digestive tract and 4 (3.39%) strains were from blood cultures of neonates with sepsis, while the number of *K. oxytoca* strains, producers of ESBLs (EPKO), was 2 (1.69%) isolated from faeces of neonates with colonization of the digestive tract (Table 1). The results obtained in this study concerning EPKP strains correlated with data recorded in investigations performed by other Bulgarian authors (VACHEVA-DOBREVSKI et al. [8]).

LEBESSI et al. [4] reported 23 colonizations and 21 infections with EPKP among neonates in NICUs at a children's hospital in Athens, Greece, diagnosed in the period of 1997–1998.

We registered 73 cases of neonates with intestinal colonizations and 4 cases of sepsis with EPKP in a period of 18 months. The four babies with sepsis at the same time had colonization of the digestive tract. Intestinal colonization with *Klebsiella*,

Enterobacter and *Serratia* (KES) strains is a crucial step to nosocomial infection [9], and we accept this standpoint with respect to *Klebsiella* spp.

Data on in vitro susceptibility of strains of *K. pneumoniae* and *K. oxytoca* to antimicrobial agents are given in Table 1. *K. pneumoniae* strains isolated from faeces of neonates showed high values of resistance to ampicillin, gentamicin and cefamandole (94.55, 89.09 and 87.27%, respectively), and to cefotaxime, ceftriaxone and ceftazidime (83.64, 81.82 and 81.82%, respectively) and low values of resistance to amikacin and piperacillin/tazobactam (13.64 and 5.45%, respectively). Resistance to amoxicillin/clavulanic acid and ceftazidime was 55.45 and 18.18%, respectively.

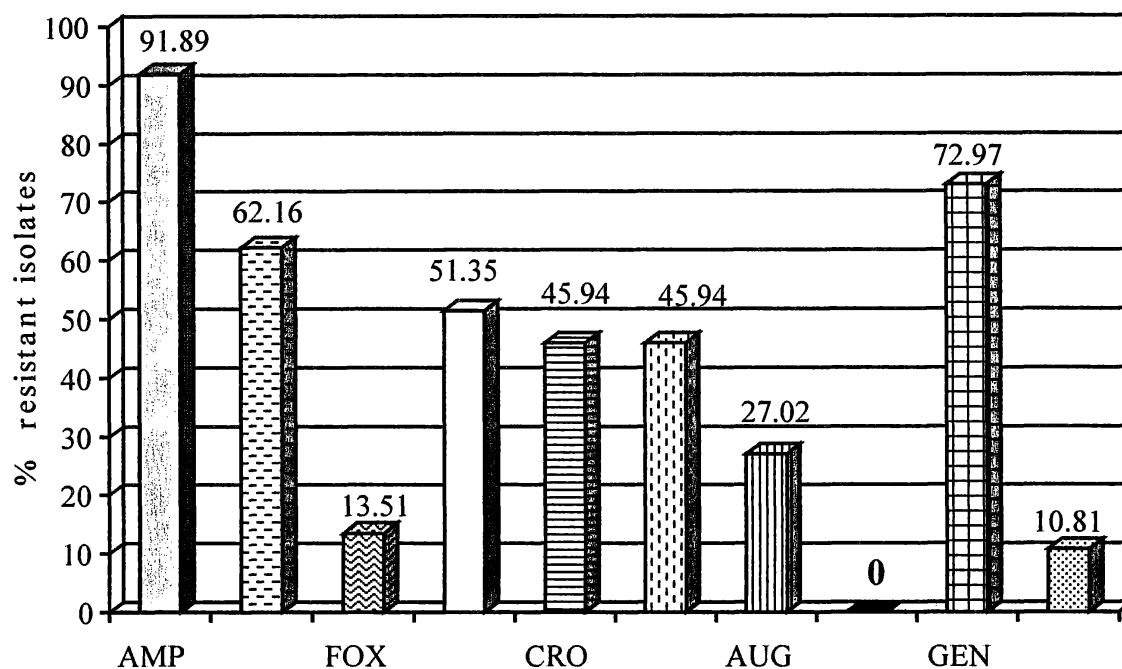


Fig. 1. Resistance to antibacterial agents of 37 *K. pneumoniae* strains isolated from faeces of new-borns with colonization of the digestive tract

Figure 1 shows the resistance to antibacterial agents of 37 *K. pneumoniae* strains isolated from faeces of neonates with colonization of the digestive tract. As it is seen, highest values of resistance were recorded to ampicillin and gentamicin (91.89 and 72.97%, respectively) and lowest ones to amikacin and piperacillin/tazobactam (10.81 and 0%, respectively).

Figure 2 shows multiple resistance to antibacterial agents of 73 EPKP strains isolated from faeces of neonates with colonization of the digestive tract. There was a 100% resistance to antibiotics of the group of cephalosporins of 2nd and 3rd generation with the exception of ceftazidime with 20.54% resistance. High values of resistance to gentamicin and ampicillin (97.26 and 95.89%, respectively) were registered which makes it impossible to use these antibiotics for empirical therapy. The lowest values of resistance to amikacin and piperacillin/tazobactam (15.06 and 8.21%, respectively) give ground to apply these two antibacterial agents to the empirical therapy of *Klebsiella*-induced colonization/infection (nosocomial infection). Both EPKO strains isolated from faeces of neonates with colonization were completely resistant to aminopenicillins and cephalosporins used in the tests, but they were susceptible to amikacin (Table 1).

ESBLs characteristics of 4 *K. pneumoniae* strains isolated from blood cultures of neonates with sepsis determines their high resistance to β -lactam antibiotics (penicillins

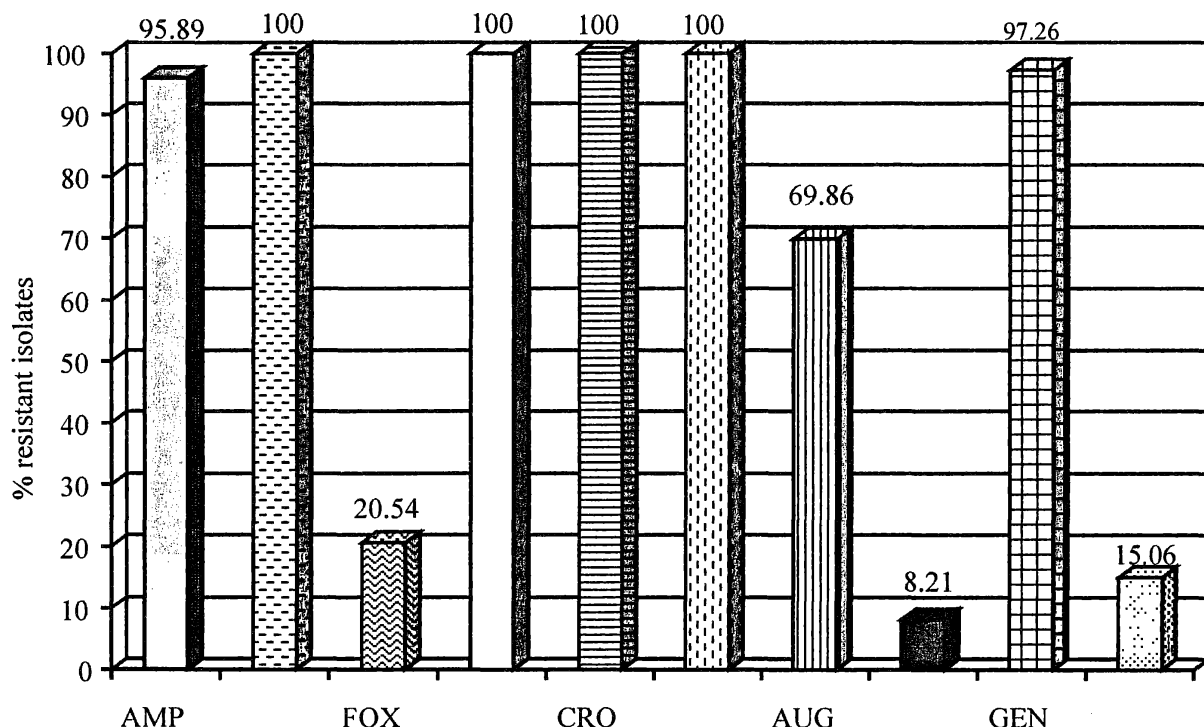


Fig. 2. Multiple resistance to antibacterial preparations of 37 strains EPKP isolated from faeces of new-borns with colonization of the digestive tract; AMP – ampicillin; MAN – cefamandole; FOX – cefoxitin; FTX – cefotaxime; CRO – ceftriaxone; CAZ – ceftazidime; AUG – amoxicillin/clavulanic acid; PPT – piperacillin/tazobactam; GEN – gentamicin; AMK – amikacin; EPKP – *K. pneumoniae* producer of extended spectrum β -lactamases

and cephalosporins), but at the same time these EPKP strains displayed high susceptibility to cefoxitin, amoxicillin/clavulanic acid, piperacillin/tazobactam, amikacin and ciprofloxacin (Table 1).

ASENSIO et al. [10] reported nosocomial outbreaks caused by multiple-resistant to 3rd generation of cephalosporins *K. pneumoniae* strains (MRKP) isolated from faeces of 10 patients at ages less than 12 weeks with infection/colonization who were treated in the paediatric intensive care unit (ICU) at a hospital in Madrid (Spain) in a period of six months (October 1997 – April 1998). MRKP strains had well-preserved susceptibility to amikacin, and this result coincides with our data.

Our results from cases of EPKP demonstrated low resistance to amikacin (15.06%) and to piperacillin/tazobactam (8.21%) (Table 1), and they correlate with data reported by other investigators [4,10,11] on these two antibacterial agents.

Monitoring of multiple resistance to antimicrobial agents of 10 of the most frequently found bacterial pathogens carried out within the frame of the European SENTRY Antimicrobial Surveillance Programme by FLUIT et al. [11] at Eijkman-Winkler Institute, University Medical Centre, Utrecht (The Netherlands), showed that *K. pneumoniae* isolates were resistant to piperacillin/tazobactam (8.9%), to ciprofloxacin (5.1%) and to amikacin (2.2%). The authors recommend that piperacillin/tazobactam, amikacin or ciprofloxacin should be used for empirical treatment, and based on our results we accept this standpoint.

Conclusion. 1. A total of 118 *Klebsiella* spp. strains isolated by us from faeces and blood cultures of neonates with colonization of the digestive tract and with sepsis belonged to two species: *K. pneumoniae* 114 (96.61%) strains, of them 110 (93.22%)

isolated from faeces of neonates with colonization and 4 (3.39%) isolated from blood culture of neonates with sepsis, and *K. oxytoca* 4 (3.39%) strains isolated from faeces of neonates with colonization.

2. Production of ESBLs in *Klebsiella* spp. was found in 79 strains (66.94%), of them 77 (65.25%) were EPKP strains. Of the latter strains 73 (61.86%) strains were isolated from faeces of new-born babies with colonization of the digestive tract, and 4 strains (3.39%) were isolated from blood culture of new-borns with sepsis. Two strains (1.69%) were EPKO that were isolated from faeces of neonates with colonization of the digestive tract.

3. The four EPKO strains causing sepsis in neonates were resistant to cephalosporins of 2nd and 3rd generation with the exception of cefoxitin, and susceptible to amoxicillin/clavulanic acid, piperacillin/tazobactam, amikacin, and ciprofloxacin.

4. Strains of *K. pneumoniae* and EPKP displayed high resistance to ampicillin (91.89 and 95.89%, respectively), cefamandole (62.16 and 100%, respectively), cefotaxime (51.35 and 100%, respectively), ceftriaxone (45.94 and 100%, respectively), cef-tazidime (45.94 and 100%, respectively), gentamicin (72.97 and 97.26%, respectively), and low resistance to amikacin (10.81 and 15.06%, respectively) and to piperacillin/tazobactam (0 and 8.21%, respectively); this gives ground to use the latter two antibacterial agents as means of choice for treatment of new-born babies with colonization and infection (nosocomial *Klebsiella* infection).

5. Empirical therapy of neonates with nosocomial *Klebsiella* infection may include amikacin and piperacillin/tazobactam, and now it is a practice at the neonatological intensive care unit of the Institute Hospital in Pleven, Bulgaria.

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Department of Microbiology and Virology
Higher Institute of Medicine
1, Kl. Okhridski Str.
5800 Pleven, Bulgaria