Nosocomial Plasmids Responsible for Multiresistance of Bacterial Isolates at Different Wards of the Children's University Hospital in Bratislava, Slovakia

M. Vrábelová[*]
K. Kollárová[*]
D. Michálková-Papajová, PhD[*]
J. Hanzen, MD[†]
P. Milošovič, MD[†]
T. Mačičková, PhD[‡]
M. Kettner, PhD[*]

[*]*Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia*
[†]*HPL Diagnostic Laboratory, Bratislava, Slovakia*
[‡]*Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia*

KEY WORDS: antibiotic resistance; *Enterobacteriaceae*; nosocomial plasmids

ABSTRACT

Susceptibility of 75 clinical isolates of *Enterobacteriaceae* to 15 aminoglycosides, beta-lactams and fluoroquinolones was studied. The isolates originated from 3 wards (Pathological neonates, Surgical ICU, and Pediatric cardiology) of the Children's University Hospital in Bratislava, Slovakia. The isolates were collected from patients in April and November 1999 and June 2001. All isolates were resistant to gentamicin, tobramycin, and ampicillin. The majority of isolates were resistant to netilmicin, amikacin, cephalosporins, and azthreonam, but they were susceptible to meropenem and fluoroquinolones. The prevailing number of isolates produced 2 to 4 aminoglycoside-modifying enzymes. All isolates produced beta-lactamases and 80% produced extended-spectrum beta-lactamases (ESBL). Plasmid analysis revealed in the majority of isolates, originating from all 3 wards, a 116 kb plasmid throughout the entire period of study. Restriction analysis suggested a dissemination and persistence of a single nosocomial plasmid at all 3 units of the large pediatric hospital in Bratislava.

INTRODUCTION

Children's hospitals serve unique patient populations with many patients having special needs. Nosocomial infections are important adverse events that complicate the hospitalization of

Table 1. Clinical Isolates of Multiresistant *Enterobacteriaceae,* Collected at 3 wards of the Children´s University Hospital, Bratislava, in April 1999 (A), in November 1999 (N), and in June 2001 (J)

No.	Microorganism	Origin	Biological material
1.	*Citrobacter freundii* 7A	Pathological neonates - PATNEO	urine
2.	*Klebsiella pneumoniae* 1A	PATNEO	urine
3.	*Klebsiella pneumoniae* 2A	PATNEO	tonsillar tampon
4.	*Klebsiella pneumoniae* 3A	PATNEO	urine
5.	*Klebsiella pneumoniae* 4A	PATNEO	urine
6.	*Klebsiella pneumoniae* 5A	PATNEO	sputum
7.	*Klebsiella pneumoniae* 14A	PATNEO	urine
8.	*Klebsiella pneumoniae* 15A	PATNEO	urine
9.	*Klebsiella pneumoniae* 16A	PATNEO	urine
10.	*Klebsiella pneumoniae* 17A	PATNEO	catheter
11.	*Klebsiella pneumoniae* 18A	PATNEO	urine
12.	*Klebsiella pneumoniae* 19A	PATNEO	pus
13.	*Klebsiella pneumoniae* 20A	PATNEO	urine
14.	*Klebsiella pneumoniae* 24A	PATNEO	tonsillar tampon
15.	*Klebsiella pneumoniae* 25A	PATNEO	pus
16.	*Citrobacter freundii* 22A	Surgical ICU - SICU	tonsillar tampon
17.	*Escherichia coli* 6A	SICU	wound
18.	*Klebsiella pneumoniae* 11A	SICU	pus
19.	*Klebsiella pneumoniae* 12A	Pediatric cardiology - CARDIO	wound
20.	*Klebsiella pneumoniae* 13A	CARDIO	wound
21.	*Klebsiella pneumoniae* 21A	CARDIO	tonsillar tampon

patients and result in considerable morbidity, mortality, and increased length of hospital stay.[1-3] Over the past several decades, the frequency of antimicrobial resistance and its association with serious diseases have increased at alarming rates. Antimicrobial resistance among gram-negative isolates is also a concern. The most important gram-negative resistance problems that impact on nosocomial infections are extended-spectrum beta-lactamases (ESBL).[4] Cross-resistance may limit the value of aminoglycosides in these types of infections. Fluoroquinolone resistance is also increasing among these ESBL strains.[5] The above mentioned enzymes are encoded by plasmids and are transferable among bacteria by means of recombination processes, particularly conjugation.

In our previous study, we observed persistence and dissemination of a unique nosocomial plasmid at several wards of the Pediatric University Hospital in Munich, Germany, during a longer time period. With a similar aim we followed, during the years 1999 to 2001, the incidence of transferable resistance of *Enterobacteriaceae* isolates at 3 different wards of the Children's University Hospital in Bratislava, Slovakia, to 8 beta-lactams, 5 aminoglycosides, and 2 fluoroquinolones. The wards included in this study were represented by Pathological neonates, Surgical ICU, and Pediatric cardiology. Impaired host defenses, invasive monitoring, exposure to multiple antibiotics, and colonization with resistant microorganisms render neonates and infants highly susceptible to nosocomial bloodstream, wound, respiratory, and urinary tract infections. We wanted therefore to study the eventual occurrence of nosocomial plasmids at another large pedi-

Table 1. Clinical Isolates of Multiresistant *Enterobacteriaceae*. (Continued)

No.	Microorganism	Origin	Biological material
1.	*Enterobacter cloacae* 55N	PATNEO	urine
2.	*Enterobacter cloacae* 61N	PATNEO	urine
3.	*Escherichia coli* 41N	PATNEO	urine
4.	*Escherichia coli* 46N	PATNEO	urine
5.	*Escherichia coli* 51N	PATNEO	catheter
6.	*Escherichia coli* 52N	PATNEO	cerebrospinal liquor
7.	*Escherichia coli* 54N	PATNEO	urine
8.	*Escherichia coli* 58N	PATNEO	urine
9.	*Escherichia coli* 59N	PATNEO	urine
10.	*Escherichia coli* 62N	PATNEO	urine
11.	*Klebsiella pneumoniae* 30N	PATNEO	tonsillar tampon
12.	*Klebsiella pneumoniae* 32N	PATNEO	blood
13.	*Klebsiella pneumoniae* 37N	PATNEO	blood
14.	*Klebsiella pneumoniae* 38N	PATNEO	tonsillar tampon
15.	*Klebsiella pneumoniae* 40N	PATNEO	urine
16.	*Klebsiella pneumoniae* 44N	PATNEO	urine
17.	*Klebsiella pneumoniae* 45N	PATNEO	urine
18.	*Citrobacter freundii* 33N	SICU	urine
19.	*Escherichia coli* 35N	SICU	nasal tampon
20.	*Escherichia coli* 48N	SICU	pus
21.	*Escherichia coli* 53N	SICU	other
22.	*Escherichia coli* 63N	SICU	urine
23.	*Klebsiella pneumoniae* 34N	SICU	nasal tampon
24.	*Klebsiella pneumoniae* 36N	SICU	tonsillar tampon
25.	*Klebsiella pneumoniae* 39N	SICU	tonsillar tampon
26.	*Klebsiella pneumoniae* 42N	SICU	tonsillar tampon
27.	*Klebsiella pneumoniae* 50N	SICU	cerebrospinal liquor
28.	*Salmonella enteritidis* 43N	SICU	tonsillar tampon
29.	*Escherichia coli* 31N	CARDIO	urine
30.	*Escherichia coli* 47N	CARDIO	urine
31.	*Escherichia coli* 65N	CARDIO	tonsillar tampon
32.	*Klebsiella pneumoniae* 49N	CARDIO	urine
33.	*Klebsiella pneumoniae* 56N	CARDIO	tonsillar tampon
34.	*Klebsiella pneumoniae* 57N	CARDIO	urine
35.	*Klebsiella pneumoniae* 60N	CARDIO	tonsillar tampon
36.	*Klebsiella pneumoniae* 64N	CARDIO	tonsillar tampon
37.	*Klebsiella pneumoniae* 66N	CARDIO	tonsillar tampon

atric hospital.

MATERIALS AND METHODS

Bacterial Strains and Susceptibility Testing

Seventy-five clinical isolates of *Enterobacteriaceae* (37 isolates collected in April 1999, 20 isolates collected in November 1999, and 18 isolates from June 2001) originating from 3 wards of the Children's University Hospital in Bratislava (Pathological neonates – PATNEO, Surgical ICU – SICU and Pediatric cardiology – CARDIO) were studied. The majority of them were isolated from urine and tonsillar tampon and the isolates were chosen on the basis of the aminoglycoside resistance.

Table 1. Clinical Isolates of Multiresistant *Enterobacteriaceae.* (Continued)

No.	Microorganism	Origin	Biological material
1.	*Enterobacter cloacae* 12J	PATNEO	tonsillar tampon
2.	*Enterobacter intermedium* 15J	PATNEO	catheter
3.	*Klebsiella pneumoniae* 20J	PATNEO	sputum
4.	*Klebsiella pneumoniae* 7J	PATNEO	urine
5.	*Klebsiella pneumoniae* 14J	PATNEO	tonsillar tampon
6.	*Enterobacter cloacae* 18J	SICU	wound
7.	*Escherichia coli* 6J	SICU	urine
8.	*Klebsiella oxytoca* 17J	SICU	wound
9.	*Klebsiella pneumoniae* 22J	SICU	thoracic drain
10.	*Klebsiella pneumoniae* 8J	SICU	urine
11.	*Klebsiella pneumoniae* 23J	SICU	wound
12.	*Escherichia coli* 3J	CARDIO	bronchoscopy
13.	*Klebsiella oxytoca* 19J	CARDIO	catheter
14.	*Klebsiella oxytoca* 10J	CARDIO	urine
15.	*Klebsiella planticola* 13J	CARDIO	urine
16.	*Klebsiella pneumoniae* 21J	CARDIO	blood
17.	*Klebsiella pneumoniae* 11J	CARDIO	urine

Susceptibility testing to following antibiotics: ampicillin (AMPI), cefoxitin (CFOX), ceftriaxone (CIAX), cefotaxime (CTAX), ceftazidime (CTAZ), cefepime (CFEP), azthreonam (AZTR), meropenem (MERO), gentamicin (GEN), tobramycin (TOB), netilmicin (NET), amikacin (AMI), isepamicin (ISE), ciprofloxacin (CIP), and ofloxacin (OFL), was performed using the agar dilution method according to NCCLS on Mueller-Hinton agar containing two-fold dilutions of antibiotic solutions ranging in concentration from 128 to 0.5 mg/L.[6]

Aminoglycoside Resistance Mechanisms

The presence of aminoglycoside-modifying enzymes (AGME) was assayed in cell-free preparations of isolates obtained by ultrasonic disruption. Enzymatic activities were measured as described previously.[7] Classification of enzymes was carried out according to the scheme by Shaw et al.[8]

Detection of Beta-Lactamases

For detection of beta-lactamase activity, the nitrocefin method was used.[9] An orange-red coloration after 30 minutes incubation was considered a positive reaction. For production of ESBL, the isolates were screened by double-disk diffusion test.[10] The enlargement of the inhibition zone between the disk containing clavulanate and that containing CTAX or CTAZ respectively, suggested the presence of ESBL.

The presence of the bla_{TEM} gene coding for TEM-type beta-lactamases was determined by a PCR method.[11] Reaction mixture for PCR was prepared as described previously.[12]

Transferability of Resistance and Plasmid DNA Study

Transferability of resistance was detected by bacterial conjugation and confirmed by isolation of plasmid DNA. Conjugation was performed with *E. coli* K12 3110 *rif* ^{r} (obtained from M. H. Richmond, UK) as described previously.[13] Plasmid DNA from donors and *E. coli* transconjugants was prepared according to this method.[14] Plasmid DNA was studied by agarose gel electrophoresis with plasmid DNA standards. For digestion of plasmid DNA by restriction endonuclease the

Table 2. Molecular Weight of Plasmid DNAs Isolated from Clinical Isolates Collected in April and November 1999 and June 2001 and Their Transconjugants

Microorganism	Origin	pDNA (KB)
Citrobacter freundii 7A	PATNEO	**116**
Escherichia coli 3110 7AT2		99
Klebsiella pneumoniae 11A	PATNEO	**116**
Escherichia coli 3110 5AT2		103
Klebsiella pneumoniae 19A	PATNEO	**116**
Escherichia coli 3110 19AT2		**116**
Citrobacter freundii 22A	SICU	**116**
Escherichia coli 3110 22AT1		108
Klebsiella pneumoniae 11A	SICU	**116**
Escherichia coli 3110 11AT1		**116**
Enterobacter cloacae 61N	PATNEO	**116**
Escherichia coli 3110 61NT2		109
Escherichia coli 46N	PATNEO	**116**
Escherichia coli 3110 46NT1		**116**
Klebsiella pneumoniae 7J	PATNEO	86; **116**
Escherichia coli 3110 7JT1		86; **116**
Escherichia coli 6J	SICU	**116**; 128; 146
Escherichia coli 3110 6JT1		**116**; 128; 146
Klebsiella pneumoniae 8J	SICU	146; 157
Escherichia coli 3110 8JT1		146; 157
Escherichia coli 3J	CARDIO	146; 151
Escherichia coli 3110 3JT1		146; 151

*Eco*RI enzyme was used.[15] After an incubation during 5 hours at 37°C digestion, profiles of the respective plasmids were studied again by agarose gel electrophoresis.

RESULTS

A list of 75 clinical isolates of *Enterobacteriaceae*, collected in April and November 1999 and June 2001 from 3 different wards (PATNEO, SICU and CARDIO), is presented in Table 1. The majority of clinical isolates represented *Klebsiella pneumoniae* (59%) and *E. coli* (24%). In vitro susceptibility of isolates to 15 antibiotics is presented in Figure 1. All isolates were resistant to GEN, TOB, and AMPI and were susceptible or nearly susceptible to MERP and fluoroquinolones during the entire period of the study. There was no resistance observed to AZTR in isolates collected in April 1999. Relatively low resistances to ISE and CTAX were also observed.

The occurrence of AGME in the isolates is shown in Figure 2. The enzymes occurring most often were APH(2´´) and AAC(6´)-III. APH(2´´) inactivates GEN, TOB, and AAC(6´)-III inactivates TOB, NET, AMI, ISE. All isolates studied were GEN and TOB resistant and majority of them were also NET resistant, although AMI resistance was also considerable, especially in November 1999.

The occurrence of beta-lactamases, TEM beta-lactamases, and ESBL enzymes is presented in Figure 3. All isolates tested, regardless of the period of their collection, produced beta-lactamase and the majority of them produced ESBL enzymes and TEM beta-lactamases, what is documented by a high rate of resistance to all groups of beta-lactams, except carbapenems.

The molecular weight of plasmids

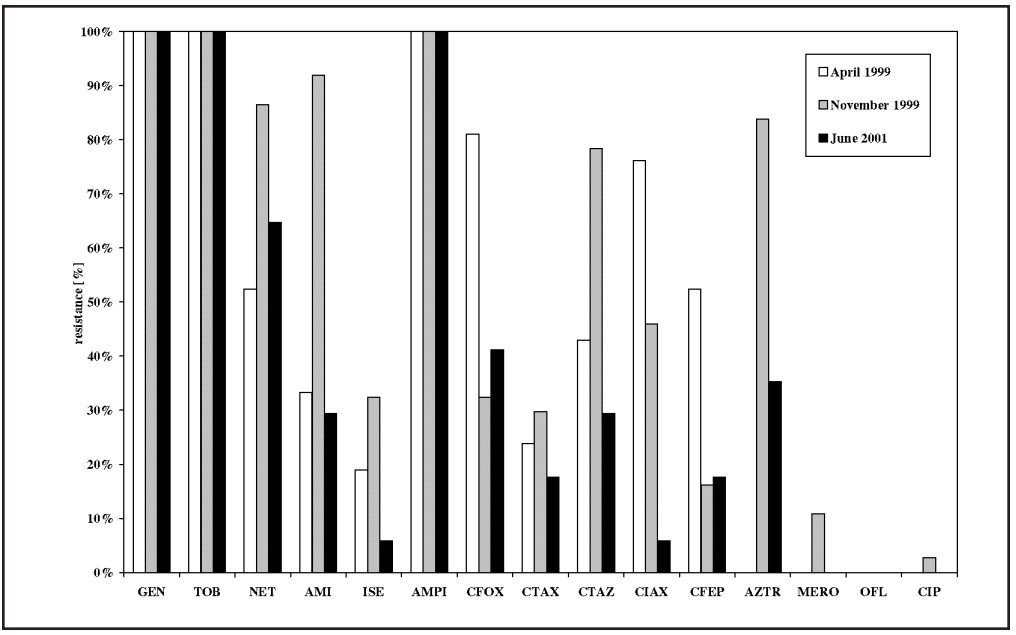

Figure 1. Resistance of clinical isolates, collected at 3 wards of the Children´s University Hospital, Bratislava, in April 1999, November 1999 and June 2001 to aminoglycosides, beta-lactams, and fluoroquinolones.

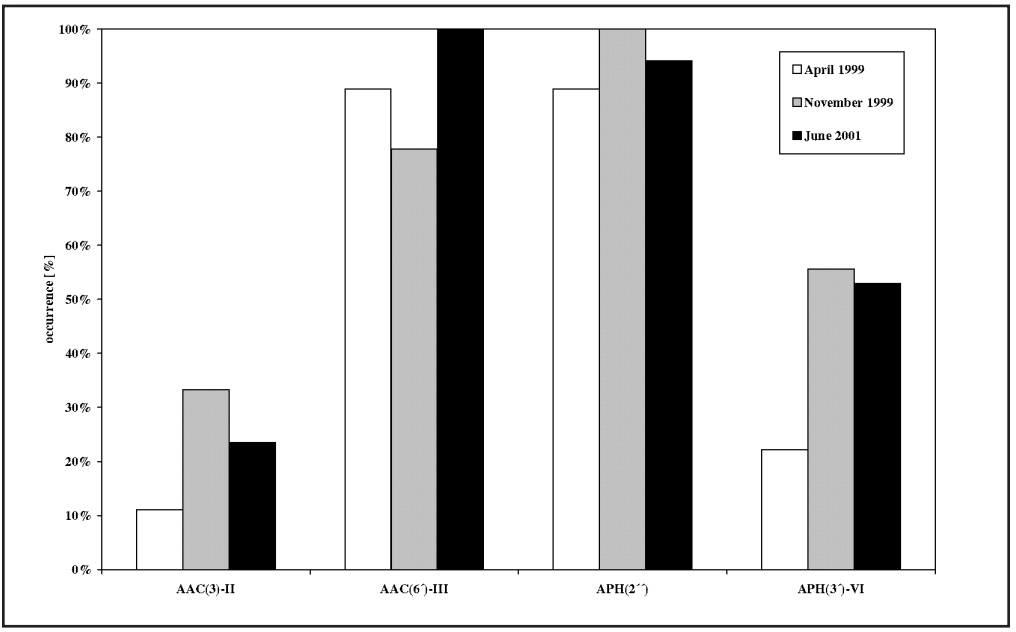

Figure 2. Occurrence of aminoglycoside modifying enzymes (AGME) in clinical isolates, collected in April 1999, November 1999, and June 2001.

isolated from clinical isolates and their transconjugants are presented in Table 2. It is evident, that in many donors and often also in their transconjugants from PATNEO and SICU wards, irrespective of the period of collection, a 116 kb plasmid occurred. Restriction profiles of such 116 kb plasmids isolated from different clinical isolates collected in 3 periods from Pathological neonates (A)

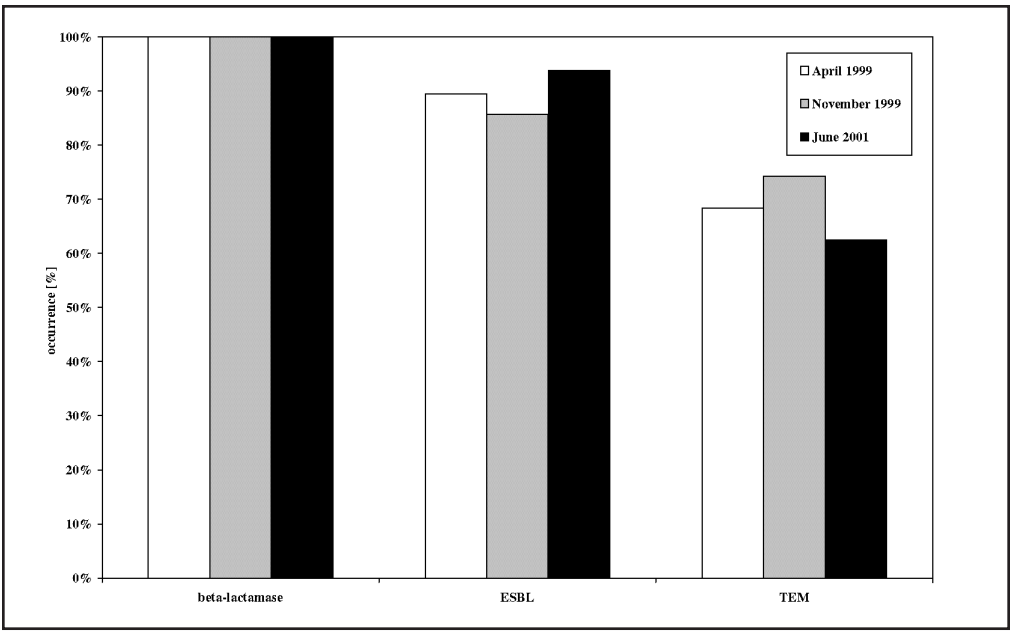

Figure 3. Occurrence of beta-lactamases, ESBL and TEM beta-lactamases in clinical isolates, collected in April 1999, November 1999, and June 2001.

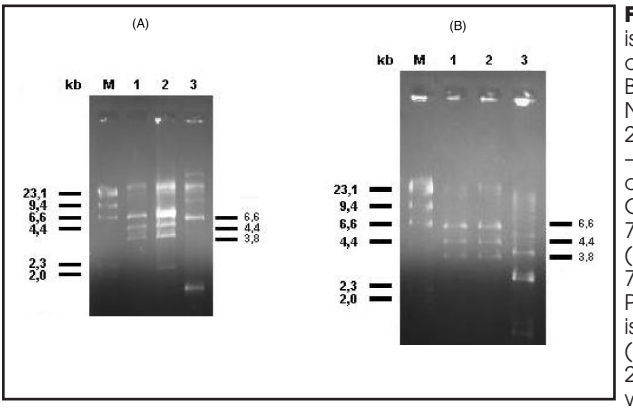

Figure 4. Plasmid DNA from clinical isolates originating from three wards of the Children´s University Hospital, Bratislava, in April 1999 (lane 1), November 1999 (lane 2) and June 2001 (lane 3), digested with EcoRI. M – DNA molecular size marker (lambda DNA digested with *Hind*III). (A) Clinical isolates *Citrobacter freundii* 7A (lane 1), *Escherichia coli* 46N (lane 2) and *Klebsiella pneumoniae* 7J (lane 3) were obtained from Pathological neonates. (B) Clinical isolates *Klebsiella pneumoniae* 11A (lane 1), *Escherichia coli* 35N (lane 2) and *Escherichia coli* 6J (lane 3) were obtained from Surgical ICU.

and Surgical ICU (B) are presented in Figure 4.

DISCUSSION

Multiple antibiotic resistance to useful classes of the antibiotics, including beta-lactams, aminoglycosides and fluoroquinolones has gradually increased among a number of Gram-negative hospital pathogens, especially *Klebsiella* spp., *Enterobacter* spp., and *E. coli*.[16] The driving force of antibiotic resistance is the widespread use of antibacterial drugs. From this point of view a good susceptibility of all isolates to meropenem and fluoroquinolones is understandable, as they were not used or very rarely used for pediatric patients where primarily beta-lactams represent antibacterials of the first choice. As the isolates were chosen on the basis of the GEN, TOB and/or NET resistance, 100% resistance to GEN and TOB and high rate of NET and AMI resistance may be explained also by the increased usage of aminoglycosides in the therapy

of infections caused by so called "problem bacteria" in Slovakia.[17] Interesting however, was observation of ISE resistance in spite of the fact that isepamicin has not been used in the therapy in Slovakia yet. But as the enzyme APH(3´)-VI has for substrates AMI and ISE, an increased usage of AMI may have supported a dissemination of this mechanism of resistance in populations of bacterial pathogens.

All clinical isolates and several transconjugants were investigated for presence of beta-lactamases and ESBL. In all isolates, during the entire period of study, the presence of beta-lactamase was observed. In more then 80% of isolates a production of ESBL and in nearly 70% of them a TEM beta-lactamases was noted. There were no substantial differences among isolates originating from different units of the pediatric hospital. Extended-spectrum beta-lactamases are now a problem in hospitalized patients worldwide. In Europe, the prevalence of ESBL production among isolates of *Enterobacteriaceae* varies greatly from country to country and from institution to institution. Across Europe, the incidence of CTAZ resistance among *K. pneumoniae* strains was 20% for non-ICU isolates and 42% for isolates from ICU patients.[18] A common reason for a widespread incidence of ESBL is a high volume and indiscriminate administration of expanded-spectrum cephalosporines.[19,20] In our study, the incidence of ESBL-producing strains prevailed in *Klebsiella* spp. ESBL are most often encoded on plasmids, which can easily be transferred between isolates. ESBL-producing *Enterobacteriaceae* have been responsible for numerous outbreaks of infections throughout the world and pose challenging infection control issues.[21]

Several authors reported that a single self-transmissible plasmid was found in isolates from numerous patients at different units of the same hospital.[22,23] Such nosocomial plasmid was responsible for causing infections or colonizations throughout the year and this result was concordant with those obtained by plasmid profiling, with slight variations. The restriction pattern indicated common DNA fragment in most plasmids isolated.[22,24] Plasmid profiling belongs to the best-suited techniques for investigating the epidemiological relatedness of strains causing nosocomial infections.

In our study, we observed the prevailing incidence of a 116 kb plasmid at all 3 hospital units throughout the 3-year period. Restriction analyses revealed identity of a single plasmid at different wards (*K. pneumoniae* 7J – PATNEO, *E. coli* 6J – SICU), but also its persistence on the same unit in different bacterial isolates and in different periods of collection (*C. freundii* 7A – April 1999, *E. coli* 46N – November 1999, *K. pneumoniae* 7J – June 2001, all PATNEO). We presumed therefore that the dissemination of a single plasmid or of several related plasmids harboring common DNA fragments in most of the isolates, occurring at different units of the University Pediatric Hospital in Bratislava, are responsible for beta-lactam and aminoglycoside resistance of *Enterobacteriaceae* isolates.

ACKNOWLEDGEMENT

The research was financially supported by the Slovak grant VEGA No. 1/8221/01.

REFERENCES

1. Ford-Jones EL, Mindorff CM, Langley JM, et al. Epidemiologic study of 4684 hospital-acquired infections in pediatric patients. *Pediatr Infect Dis J.* 1989;8:668-675.
2. Appelgren P, Hellström I, Weitzberg E, Söderlund V, Bindslev V, Ransjö U. Risk factors for nosocomial intensive care infection: a long-term prospective analysis. *Acta Anaesthesiol Scand.* 2001;45:710-719.
3. Stover BH, Shulman ST, Bratcher DF, Brady MT, Levine GL, Jarvis WR. Nosocomial

infections rates in US children's hospitals´ neonatal and pediatric intensive care units. *Am J Infect Control.* 2001;29:152-157.

4. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology and detection of this important resistance threat. *Clin Microbiol Rev.* 2001;14:933-951.

5. Jones RN. Resistance patterns among nosocomial pathogens. *Chest.* 2001;119:397S-404S.

6. National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. *Approved standard M7-A5*; 2000. Wayne, Penn.

7. Kettner M, Navarová J, Ryďl Z, Knothe H, Lebek G, Krěméry V. Occurrence of aminoglycoside-modifying enzymes in resistant strains of enterobacteria and *Pseudomonas aeruginosa* from several countries. *J Antimicrob Chemother.* 1981;8:175-185.

8. Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev.* 1993;57:138-163.

9. O´Callaghan CH, Morris A, Kirby SM, Shingler AH. Novel method for detection of beta-lactamase by using a chromogenic cephalosporin substrate. *Antimicrob Agents Chemother.* 1972;1:283-288.

10. Jarlier V, Nicolas M, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis.* 1988;10:867-878.

11. Stapleton P, Wu PJ, King A, Shannon K, French G, Phillips I. Incidence and mechanisms of resistance to the combination of amoxicillin and clavulanic acid in *Escherichia coli*. *Antimicrob Agents Chemother.* 1995;39:2478-2483.

12. Vrábelová M, Černická J, Kvačkajová J, et al. Occurrence of aminoglycoside-modifying enzymes and beta-lactamases in *Acinetobacter* spp., isolated from different hospitals in Bratislava. *Biologia.* 2003;58:1087-1094.

13. Kettner M, Milošovič P, Hletková M, Kallová J. Incidence and mechanism of aminoglycoside resistance in *Pseudomonas aeruginosa* serotype O11 isolates. *Infection.* 1995;23:380-383.

14. Takahashi S, Nagano Y. Rapid procedure for isolation of plasmid DNA and application to epidemiological analysis. *J Clin Microbiol.* 1984;20:608-613.

15. Qiagen. Qiagen Plasmid Purification Handbook. 2000;09:16-19.

16. Struelens MJ. The epidemiology of antimicrobial resistance in hospital-acquired infections: problems and possible solutions. *British Med J.* 1998;317:652-654.

17. Milošovič P, Mačičková T, Kettner M, Kallová J. Development of amikacin resistance in bacterial isolates in Slovakia (1990-1992). *Int J Antimicrob Agents.* 1994;4:69-71.

18. Bradford PA, Cherubin CE, Idemyor V, Rasmussen BA, Bush K. Multiply resistant *K. pneumoniae* strains from two Chicago Hospitals: identification of the extended-spectrum TEM-12 and TEM-10 ceftazidime-hydrolyzing beta-lactamases in a single isolate. *Antimicrob Agents Chemother.* 1994;38:761-766.

19. Peňa C, Pujol M, Ricart A, et al. Risk factors for fecal carriage of *Klebsiella pneumoniae* producing ESBL in the intensive care unit. *J Hosp Infect.* 1997;35:9-16.

20. Rice LB. Successful intervention for Gram-negative resistance to extended-spectrum beta-lactam antibiotics. *Pharmacotherapy.* 1999;19:1205-1285.

21. Rupp ME, Fey PD. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* consideration for diagnosis, prevention and drug treatment. *Drugs.* 2003;63:353-365.

22. Araque M, Nieves B, Ruiz O, Dagert M. Characterization of plasmids which mediate resistance to multiple antibiotics in Gram-negative bacteria of nosocomial origin. *Enferm Infec Microbiol Clin.* 1997;15:299-305.

23. Marchandin H, Carriere C, Sirot D, Jean-Pierre H, Darbas H. TEM-24 produced by four different species of *Enterobacteriaceae,* including *Providencia rettgeri,* in a single patient. *Antimicrob Agents Chemother.* 1999;43:2069-2073.

24. Jalaluddin S, Devaster J, Scheen R, Gerard M, Butller JP. Molecular epidemiological study of nosocomial *Enterobacter aerogenes* isolates in a Belgian Hospital. *J Clin Microbiol.* 1998;36:1846-1852.