Polish Journal of Microbiology
2004, Vol. 53, No 1, 27–34

Incidence of Extended-Spectrum β-Lactamases in Clinical Isolates of the Family *Enterobacteriaceae* in a Pediatric Hospital

RADOSZ-KOMONIEWSKA HALINA[1], GNIADKOWSKI MAREK[2], ROGALA-ZAWADA DANUTA[1], NOWAKOWSKA MARIA[1], RUDY MARIA[1], WIECHUŁA BARBARA[1] and MARTIROSIAN GAYANE[1]

[1]Department of Microbiology, Medical University of Silesia
[2]National Institute of Public Health in Warsaw

Received 19 December 2003

Abstract

The incidence of extended-spectrum β-lactamases (ESBLs) was analyzed in *Enterobacteriaceae* population circulating in the Upper Silesian Child and Mother Health Center in Katowice (USC&MHC). Altogether 1164 clinical specimens, collected from children hospitalized in 8 different hospital units of USC&MHC were investigated. Five hundred and eighty-five clinical isolates of the family *Enterobacteriaceae* were identified in specimens collected from 403 patients. Two hundred and twenty-nine *Enterobacteriaceae* strains (39%) isolated from 162 patients were found to be putative ESBL producers as revealed by double-disc synergy (DDS) test. ESBL activity was the most prevalent in the population of *Klebsiella pneumoniae* (77%), followed by *Klebsiella oxytoca* (50%), *Serratia marcescens* (43%), *Escherichia coli* (30%), *Enterobacter* spp. (18%) and *Proteus mirabilis* (12%). ESBL producers demonstrated also wide resistance to the non-β-lactam antimicrobial co-trimoxazole (93%) and the aminoglycosides netilmicin (88%), gentamicin (84%) and amikacin (79%).

Key words: ESBL, *Enterobacteriaceae*, infections, epidemiology

Introduction

Infections caused by organisms of the family *Enterobacteriaceae* nowadays pose a serious clinical problem, which, among others, is due to the spread of many new drug resistance mechanisms in these bacteria. The most common mechanism of their resistance to β-lactam antibiotics is the production of diverse β-lactamases (Livermore, 1995; Medeiros, 1997; Gniadkowski, 1997) out of which the so-called extended spectrum β-lactamases (ESBLs) belong to those of the highest clinical and epidemiological importance. They are able to hydrolyze almost all penicillins, cephalosporins (excluding cephamycins) and monobactams (Livermore, 1995; Medeiros, 1997; Gniadkowski, 1997; Bradford, 2001; Sirot, 1995). Even if ESBL-producing strains may demonstrate susceptibility *in vitro* to some of the ESBL substrates, such drugs have been often found unsuccessful in therapy (Livermore, 1995; Bradford, 2001; Paterson *et al*., 2001; Sanders *et al*., 1996). This requires clinical microbiology laboratories to routinely detect ESBLs in *Enterobacteriaceae* isolates and report them as resistant to all compounds that belong to the ESBL substrate spectrum (Livermore, 1995; Bradford, 2001; Paterson *et al*., 2001; Sanders *et al*., 1996; Steward *et al*., 2001). Therapeutic options include aminoglycosides, fluoroquinolones, co-trimoxazole, combinations of β-lactams with β-lactamase inhibitors and carbapenems. However, except for carbapenems, resistance to these drugs has been observed in more and more ESBL-producing strains in recent years (Livermore, 1995; Bradford, 2001).

ESBL-encoding genes appear due to either mobilization of chromosomal genes coding for certain species-specific β-lactamases with ESBL activity (Navarro and Miró, 2002) or, more often, due to mutations in

[1] Correspondence address: Halina Radosz-Komoniewska, Katedra i Zakład Mikrobiologii Śląskiej Akademii Medycznej, 40-752 Katowice, ul. Medyków18, tel./fax. 032/2526075, e-mail: hradosz@slam.katowice.pl

plasmid genes encoding broad-spectrum penicillinases, such as TEM-1, TEM-2 or SHV-1 (Livermore, 1995; Bradford, 2001; Gniadkowski, 2001). The extremely wide distribution of these enzymes in nosocomial *Enterobacteriaceae* populations and the strong pressure of the use of newer generation β-lactams cause that ESBL-producing strains are frequently selected *de novo* in therapy (Livermore, 1995; Gniadkowski, 2001). Once selected, ESBL producers may quickly spread in a hospital environment by clonal dissemination. Moreover, since ESBL genes are usually plasmid-located, they may rapidly penetrate the enterobacterial population by the horizontal transfer of plasmids (Livermore, 1995; Bradford, 2001; Gniadkowski, 2001). Since 1983, when the first ESBL-producing strains were identified, these enzymes have been described in many gram-negative species, however, they are still mainly observed in *Klebsiella pneumoniae* and *Escherichia coli* (Livermore, 1995; Medeiros, 1997; Gniadkowski, 1997; Bradford, 2001; Sirot, 1995). The number of known ESBL variants has been constantly growing over the period of the last 20 years, exceeding the value of 150 within 10 different enzyme families, and resulting in their high structural and biochemical diversity (Bradford, 2001; Gniadkowski, 2001).

The frequency of infections caused by ESBL producers varies depending on a group of examined patients and hospital unit specificity, which are factors that influence directly the level of antibiotic consumption (Livermore, 1995). Usually, the highest prevalence of ESBL-producing isolates within a hospital is observed in intensive care units (ICUs), neonatal and surgical wards (Gniadkowski, 2001). This study presents an analysis of *Enterobacteriaceae* isolates that were identified as putative ESBL producers in the Upper Silesian Child and Mother Health Center (USC&MHC) in Katowice in 1999–2000.

Experimental

Materials and Methods

Clinical isolates. Altogether 1164 clinical specimens, collected from newborns and children hospitalized in eight different wards of USC&MHC in Katowice, were investigated in the Department of Microbiology of the Silesian Medical University between February 1999 and September 2000. Out of all 1356 clinical isolates recovered, 709 isolates were classified into different species of the family *Enterobacteriaceae*, which constituted 52% of all the organisms identified. They were collected from 630 clinical specimens. The isolates were recovered mostly from urine (585 isolates, 82.5%) and blood (53 isolates, 7.5%), collected mainly from patients in the nephrology outpatient clinic (222 isolates, 31%), neonatal intensive care unit (197 isolates, 28%) and the pediatrics department (90 isolates, 13%). The isolates cultured from urine, blood, cerebrospinal fluid and wound swabs (662 isolates, 93.4%) were assumed to represent etiologic agents of infections, and those recovered from other materials (*e.g.* pharyngeal and nasal swabs) were interpreted as colonisers. The 630 enterobacteria-positive clinical specimens were obtained from 403 patients, the majority of which were infected or colonized with a single enterobacterial species (338 patients). Two hundred and seventy three patients were examined only once; the remaining patients (130 patients) were examined two times or more, either in terms of different kinds of specimens (10 patients) or the sequential analysis of the same specimen type (120 patients) per patient. The change of the isolated species with time was observed in the cases of 50 patients. All repeated isolates of the same bacterium from the same patient and the same sampling site were excluded from statistical analysis. The microbiological analysis of the specimens collected was conducted in accordance with routine diagnostic procedures (ATB ID32E and ID32GN, bioMérieux).

Detection of ESBL activity. ESBL activity was detected in *Enterobacteriaceae* isolates by the double disc synergy (DDS) test, using discs with amoxicillin and clavulanic acid (20/10 μg), cefotaxime (30 μg), ceftazidime (30 μg), and aztreonam (30 μg) (Becton Dickinson) (Jarlier *et al.*, 1988; Gniadkowski *et al.*, 1996). The cefotaxime, ceftazidime and aztreonam discs were placed in a distance of 20 mm (center-to-center) from the disc containing clavulanate. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 was used as positive and negative controls. A DDS test result was regarded as positive when the inhibition zone around any of the cefotaxime, ceftazidime or aztreonam discs was expanded with amoxicillin and clavulanate.

Selected isolates were also examined using E-Test ESBL strips (AB Biodisk), according to the manufacturer's instructions (ETM, 2000). An isolate was regarded as ESBL positive when it was characterized by ceftazidime MIC of at least 1 μg ml^{-1} or cefotaxime MIC of at least 0.5 μg ml^{-1}, and the ratio between cephalosporin MIC to the of its combination with clavulanate was at least 8 (Cormican *et al*., 1996; Livermore and Brown, 2001).

Antimicrobial susceptibility testing. Antimicrobial susceptibility of putative ESBL-producing isolates was tested by the disc diffusion method in accordance with NCCLS guidelines (NCCLS, 2000). The following antimicrobial discs were tested: ampicillin (10 μg), amoxicillin with clavulanic acid (20/10 μg), piperacillin (100 μg), piperacillin with tazobactam (100/10 μg), cephalothin (30 μg), cefuroxime (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), aztreonam (30 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), netilmicin (30 μg), tetracycline (30 μg), norfloxacin (10 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg) and nitrofurantoin (300 μg) (Becton Dickinson, USA). *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used as reference strains. Results were interpreted according to the NCCLS criteria (NCCLS, 2000a).

MICs of β-lactam antibiotics were also determined by the agar dilution method, in accordance with NCCLS guidelines ((NCCLS, 2000b). The following antibiotics were used: ampicillin (Polfa Tarchomin), cefuroxime (Glaxo Wellcome), ceftazidime (Glaxo Wellcome), cefotaxime (Polfa Tarchomin), cefoxitin (Sigma Chemical Company), aztreonam (Bristol Myers Squibb), imipenem (Merck Sharp & Dohme) and lithium clavulanate (SmithKline Beecham). In all clavulanate combinations the constant inhibitor concentration was 2 μg ml^{-1}.

Results

Clinical isolates. Clinical data regarding the *Enterobacteriaceae* isolates are shown in Tables I and II. The results of species identification are presented in Table III. The most prevalent were isolates of *E. coli* (303 isolates, 52%), *K. pneumoniae* (127 isolates, 22%), *Proteus mirabilis* (41 isolates, 7%), *K. oxytoca* (32 isolates, 5,5%), *Enterobacter* spp. (28 isolates, 5%) and *Serratia marcescens* (21 isolates, 3,6%). Other *Enterobacteriaceae* species were identified less frequently. Interestingly, whereas the biggest number of *E. coli* isolates were collected from patients in the nephrology outpatient clinic (94 isolates, 31%), the majority *K. pneumoniae* isolates were recovered from patients in the neonatal intensive care unit (53 isolates, 42%).

Putative ESBL-producing isolates and their clinical background. All 585 *Enterobacteriaceae* isolates were tested for the presence of ESBL activity and 229 isolates of these (39%), recovered from 162 patients, were identified as putative ESBL producers based on the positive result of the DDS test. The most frequent ESBL producer species was *K. pneumoniae*; 43% (n = 98) of all ESBL-producing isolates belonged to this species, and ESBL activity was found in 77% of all *K. pneumoniae* isolates collected. The high rate of DDS test positive results was also revealed among *K. oxytoca* (50%), *S. marcescens* (43%), *E. coli* (30%), *Enterobacter* spp. (18%) and *P. mirabilis* (12%) isolates. ESBL production was observed in four isolates of *Citrobacter freundii* (n = 11) and two isolates of *Morganella morganii* (n = 11).

Table I
The relationship of ESBL-positive/ESBL-negative strains of *Enterobacteriaceae* isolated in different wards of the USC& MHC

Hospital units	Number of *Enterobacteriaceae*		
	DDS test negative	DDS test positive (putative ESBL producers)	Total
Neonatal intensive care unit	61	101	162
Pediatrics	31	49	80
Nephrology outpatient clinic	153	14	167
Surgery department	20	14	34
Department of developmental age neurology	7	15	22
Urology outpatient clinic	18	5	23
Department of daily diagnostics and therapy	11	6	17
Department of infantile endocrinology and diabetology	4	7	11
Other	51	18	69
Total	356	229	585

Table II
The relationship of ESBL-positive/ESBL-negative strains of *Enterobacteriaceae* isolated in different clinical specimens

Material	Number of *Enterobacteriaceae*		
	DDS test negative	DDS test positive (putative ESBL producers)	Total
Urine	303	172	475
Blood	18	29	47
Cerebrospinal fluid	2	5	7
Pharyngeal swab	1	9	10
Nasal swab	1	1	2
Vaginal swab	10	1	11
Intravascular catheter	4	2	6
Wound swab	8	2	10
Other	9	8	17
Total	356	229	585

Table III
Detection of putative ESBL-producing strains by DDS test among different species of *Enterobacteriaceae*

Species	Number of strains		Number of examined strains/Percentage of ESBL-producing strains
	DDS test negative	DDS test positive (putative ESBL producers)	
Klebsiella pneumoniae	29	98	127 / 77
Klebsiella oxytoca	16	16	32 / 50
Serratia spp.	12	9	21 / 43
Citrobacter freundii	7	4	11 / 36
Escherichia coli	213	90	303 / 30
Enterobacter spp.	23	5	28 / 18
Proteus mirabilis	36	5	41 / 12
Morganella morganii	9	2	11 / 18
Other	11	0	11 / –
Total	356	229	585 / 39

One hundred and seventy-two putative ESBL-producing isolates were recovered from urinary tract infections, which constituted 75% of all ESBL producers identified and 36% of all *Enterobacteriaceae* isolates collected from urine. The higher frequency of the ESBL-producing isolates regarding the infection site was observed in the case of isolates recovered from blood (62% of which /n = 47/ demonstrated positive DDS test). The ESBL prevalence was also very high (71%) among the isolates cultured from the CSF, however, the number of all isolates obtained from this source was low (n = 7).

The majority of putative ESBL-producing isolates (101 isolates, 44%) were collected from patients hospitalized in the neonatal ICU of the USC&MHC, followed by the isolates from the pediatrics department (49 isolates, 21%). 90% and 96% of *K. pneumoniae* isolates were DDS test positive, respectively. These rates were also very high in the case of *E. coli* isolates, reaching values of 54% and 44%, respectively. Although the majority of all *Enterobacteriaceae* isolates were collected from patients in the nephrology outpatient clinic, a relatively low rate of ESBL production was observed among them (14 isolates, 6%). Thirty-nine percent of *K. pneumoniae* (n = 18) and 4% of *E. coli* (n = 94) isolates from this ward showed ESBL activity.

Antimicrobial susceptibility testing of ESBL-producing isolates. All putative ESBL-producing isolates were resistant to ampicillin, piperacillin, cephalothin and cefuroxime. A high percentage of the isolates were resistant to co-trimoxazole (93%) and aminoglycosides, including netilmicin (88%), gentamicin (83%) and amikacin (79%). Resistance to tetracycline was demonstrated in 61% of the isolates, to piperacillin with tazobactam in 23% of the isolates, and to nitrofurantoin – in 33% of urine-derived isolates. A small fraction of the isolates were resistant to norfloxacin (2%) and none of them was resistant to imipenem.

Resistance to cefotaxime and ceftazidime revealed a remarkable degree of diversity in the analyzed group of isolates, both in terms of resistance levels an patterns of resistance to the two cephalosporins. According to the standard NCCLS breakpoint criteria used in susceptibility testing, there were isolates resistant or susceptible *in vitro* to both compounds (45 and 4 isolates, respectively), as well as isolates representing almost all other possible combinations of resistance phenotypes. The most prevalent were isolates resistant to cefotaxime and susceptible to ceftazidime (69 isolates). In general resistance to cefotaxime was more widely spread among the isolates than that to ceftazidime.

Detection of ESBL activity by E-Test. Sixteen *K. pneumoniae* and 4 *E. coli* DDS-positive isolates recovered from urine, blood and CSF samples from patients in the neonatal intensive care unit were selected for ESBL detection using E-Test ESBL. The results are shown in Table IV. All these isolates revealed high-level resistance to cefotaxime with MICs exceeding the highest antibiotic concentration available in the E-Test strip (MICs, >16 μg ml^{-1}). In 14 isolates clavulanic acid reduced the cefotaxime MICs efficiently enough to indicate ESBL activity. In the remaining six isolates the results was non-interpretable, because cefotaxime with clavulanate MICs also exceeded the highest value in the test (MICs, >1 μg ml^{-1}). Ceftazidime MICs surpassed the concentration range of the test in the case of 11 isolates (MICs, >32 μg ml^{-1}). In the remaining isolates MICs of ceftazidime were between 8 and 24 μg ml^{-1}. Decrease of ceftazidime MICs in the presence of clavulanate indicated ESBL activity in 15 isolates and in five isolates the interpretation of results was not possible (ceftazidime with clavulanate MICs, >4 μg ml^{-1}). The combination of

Table IV
Detection of ESBL producers by E test ESBL among selected DDS positive strains

Compound[a]	E.c. 621	K.p. 1100	K.p. 1098	E.c. 956	K.p. 652	K.p. 797	K.p. 1457	K.p. 1367	E.c. 1337	E.c. 1158	K.p. 551	K.p. 552	K.p. 1085	K.p. 1135	K.p. 1308	K.p. 1141	K.p. 1331	K.p. 1326	K.p. 1049	K.p. 933	K.p. ATCC 700603 ESBL+	E.c. ATCC 32518 ESBL–
CT	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	1.0	<0.25
CTL	>1	0.125	0.25	>1	0.125	0.064	>1	1	>1	>1	0.25	>1	0.25	0.125	0.125	0.25	0.25	0.38	0.125	0.38	0.094	0.047
CT/CTL	ND[b]	>128	>64	ND	>128	>250	ND	>16	ND	ND	>64	ND	>64	>128	>128	>64	>64	>42	>128	>42	>10	<5
TZ	>32	>32	8	>32	8	*[c]	>32	>32	>32	>32	>32	>32	>32	>32	16	16	24	12	16	24	>32	<0.5
TZL.	>4	>4	0.25	>4	0.5	0.38	2	1	>4	>4	0.5	1	1.5	1.5	0.75	1	0.75	0.25	0.5	1	0.38	0.094
TZ / TZL	ND	ND	32	ND	16	>8	>16	>32	ND	ND	>64	>32	>21	>21	21	16	32	48	32	24	>84	<5

[a] – abbreviations: CT – cefotaxime; CTL – cefotaxime plus clavulanic acid; TZ – ceftazidime; TZL – ceftazidime plus clavulanic acid
[b] – ND; non determinable
[c] – *; deformation of the TZ inhibition ellipse

Table V
Detection of MIC of β-lactam antibiotics by agar dilution method among selected DDS-positive strains of *Enterobacteriaceae*

Antibiotic μg/ml^{-1}	E.c. 621	K.p. 1100	K.p. 1098	E.c. 956	K.p. 652	K.p. 797	K.p. 1457	K.p. 1367	E.c. 1337	E.c. 1158	K.p. 551	K.p. 552	K.p. 1085	K.p. 1135	K.p. 1308	K.p. 1141	K.p. 1331	K.p. 1326	K.p. 1049	K.p. 933	K.p. ATCC 700603	E.c. ATCC 25922
Ampicillin	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	1024	4
Cefuroxime	>2048	>2048	>2048	1024	>2048	1024	>2048	>2048	1024	1024	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	>2048	512	32	4
Cefoxitin	256	8	8	256	8	2	8	16	128	128	16	16	8	8	8	8	16	8	8	128	16	2
Cefotaxime	32	128	128	16	128	16	128	256	16	16	128	256	256	256	256	256	256	256	256	16	–	–
Cefotaxime +clavulanate	8	0.5	0.5	8	0.5	0.06	0.5	0.5	8	8	4	2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	4	0.25	0.06
Ceftazidime	32	16	32	32	16	16	16	32	32	32	256	128	16	32	16	16	32	32	32	32	32	0.125
Ceftazidime + clavulanate	16	2	2	16	2	0.25	2	2	16	16	2	2	2	4	2	2	4	2	2	16	1	0.125
Aztreonam	64	128	128	64	128	4	128	128	64	64	512	512	128	128	128	128	128	128	128	64	0.06	0.125
Aztreonam + clavulanate	32	0.5	0.5	16	0.5	≤0.03	0.5	0.5	16	16	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	16	32	0.125	0.125
Imipenem	0.5	0.125	0.125	0.5	0.125	0.125	0.125	0.125	0.5	0.5	0.125	0.125	0.125	0.25	0.125	0.125	0.25	0.125	0.125	0.125	0.25	0.125

cefotaxime and ceftazidime data demonstrated ESBL activity in 16 isolates. In four *E. coli* isolates the results were non-interpretable.

Detection of MIC of β-lactam antibiotics among E-Test ESBL positive isolates. MICs of a representative set of β-lactam antibiotics were evaluated for the 20 ESBL-producing *K. pneumoniae* and *E. coli* isolates, which had been analyzed before by the E-Test ESBL. The results are shown in Table V. Fourteen isolates were resistant to cefotaxime (MICs, 64–512 μg ml^{-1}), 13 isolates to ceftazidime (MICs, 32–256 μg ml^{-1}) and 19 isolates to aztreonam (MICs, 64–512 μg ml^{-1}). Clavulanic acid efficiently reduced MICs of the investigated antibiotics except for the four *E. coli* isolates studied that were non-interpretable in the E-Test ESBL. In the case of these isolates the decrease of cefotaxime, ceftazidime and aztreonam MICs in the presence of clavulanate was at the level of one or two consecutive dilutions. Five isolates were resistant to cefoxitin (MICs, 128–256 μg ml^{-1}), and they included the four *E. coli* isolates.

Discussion

In this work the prevalence of ESBL-producing *Enterobacteriaceae* in the USC&MHC hospital in Katowice was demonstrated. The hospital is a new large tertiary medical center specializing in gynecology, obstetrics, neonatology and pediatrics. Due to several factors, including the high representation of patients with numerous infection risk factors and high consumption of antibiotics, such hospitals are usually exposed to the danger of efficient selection and rapid spread of microorganisms with diverse antimicrobial resistance mechanisms. The number of 229 *Enterobacteriaceae* isolates from 162 patients, including 98 *K. pneumoniae* isolates that were identified as putative ESBL producers over the period of 20 months seems to be very high. It may be compared only to a few cases described previously, like in a ~500 bed hospital in New York in the beginning of the 1990s, where 155 patients were infected or colonized with ESBL-producing *K. pneumoniae* over 19 months (Meyer *et al*., 1993). In 1993–95, 145 patients of a ~1,000 bed hospital in Barcelona were infected or colonized by ESBL-producing *K. pneumoniae* within a period of 25 months (Peña *et al.*, 1998).

The putative ESBL-producing strains have strongly contributed to nosocomial populations of almost all *Enterobacteriaceae* species identified. The highest rate of ESBL production, both in terms of the total number of ESBL producers as well as their percentage among all isolates of a species recovered at the time, was found in the case of *K. pneumoniae.* The major role of this species in ESBL production has been widely observed from the time of the first ESBL isolations (Livermore, 1995; Bradford, 2001; Gniadkowski, 2001, Bush, 1996). However, the rate of 77% of putative ESBL producers among all *K. pneumoniae* isolates in the USC&MHC at the time of the study seems to represent one of the more extreme cases ever reported and this value was even higher in certain wards (more than 90%), such as the neonatal intensive care unit and the pediatrics department. Similar data on the very high representation of ESBL-expressing *K. pneumoniae* isolates in hospitals or hospital wards may be found in other reports from Polish medical centers. For example, Kędzierska *et al.* (1999) reported a number of 91% of ESBL producers among *K. pneumoniae* recovered from patients in the neonatal ICU and the prematurity department at the Neonatology University Hospital in Cracow. Lower, though still very high rates were documented by Andrzejewska *et al.* (1998) (40.4%) and Drulis – Kawa *et al.* (2000) (32.5%), whereas the value reported by Pałucha *et al.* (1999) in one hospital in Warsaw (16%) is located within ranges that are usually observed in Europe and the USA. In the study by Babini and Livermore (2000), who examined klebsiellae isolates from 21 ICU in Western and Southern Europe within the years 1997–98, the prevalence of ESBL-producing strains was estimated at the level of 25%. The representation exceeding 30% occurred sporadically in particular units in Turkey (even 83%), France (62%), Italy (52%), Belgium (37%) and Germany (34.5%).

Also noteworthy is the very high prevalence of putative ESBL producers in the *E. coli* population in the USC&MHC, reaching the value of 30% of all isolates of the species analyzed. Although in general *E. coli* has been often observed as an important host of ESBL expression, it was usually due to high total number of this organism among ESBL producers in survey studies and not to the proportion of ESBL positive strains to all *E. coli* isolates. It is possible that a certain fraction of DDS test positive results among the USC&MHC *E. coli* isolates were false positive (see below), however, still the possible frequency of ESBL production in this species seems to be very high. In works of other authors, the frequency of ESBL occurrence in *E. coli* ranged from 1,69% to 10% (Janicka *et al*., 1997; Kędzierska *et al.*, 1999; Andrzejewska *et al.*, 1998; Drulis-Kawa *et al.*, 2000; Saurina *et al*., 2000). The prevalence of ESBL-producing *P. mirabilis* (12%) in the USC&MHC was significantly lower than that reported by Szymaniak *et al.* (1999) in a hospital in Szczecin (59%) and comparable to data obtained in a large survey conducted in France by De Champs *et al.* (2000)

(6.9%). In last years, the increasing role of AmpC producer species, such as *Enterobacter* spp., *C. freundii*, *S. marcescens* and *M. morganii*, has been observed among ESBL-expressing strains in different countries, *e.g.* Argentina and France (Gniadkowski, 2001). This phenomenon could also be noticed in the USC&MHC populations of *Enterobacteriaceae*, and, especially in *S. marcescens*, in which the prevalence of ESBL was estimated at the level of 43%. Similarly high values of the prevalence of ESBL-producing *S. marcescens* were reported in Poland before by Ulatowska *et al.* (2000) (57.7%), Pałucha *et al.* (1999) (32%), and Kędzierska *et al.* (1999) (25%).

The distribution of ESBL-producing isolates with respect to particular wards of the hospital is similar to the situation observed in other hospitals in many countries (Livermore, 1995; Gniadkowski, 2001). The majority of the isolates (44%) were recovered from patients in the neonatal ICU. Due to many factors, these patients form a group of a high risk of nosocomial infection and are intensively treated with antibiotics, which, moreover, creates an additional, strong infection risk factor. The very high incidence of putative ESBL producers in *Enterobacteriaceae* populations in the pediatrics department (96% of *K. pneumoniae*, 44% of *E. coli*) is striking, even if the total number of isolations was significantly lower in this ward than in the neonatal ICU (21% of all putative ESBL producers). This observation stimulates further epidemiological studies in this ward. What is also noteworthy is the high percentage of ESBL-producing strains among the isolates from blood. Sixty-two percent of *Enterobacteriaceae* isolates from blood showed ESBL activity. Moreover, 5 out of 7 *Enterobacteriaceae* isolates from CSF demonstrated the presence of these enzymes. In the work of Wojsyk-Banaszak *et al.* (2000) 62.9% of multi-resistant strains of *K. pneumoniae* with ESBL expression (17 of 27) were the etiologic agents of infections of the central nervous system in newborns.

In the study by Babini and Livermore (2000) mentioned above on ESBL-producing klebsiellae from 21 intensive care units in Europe, 61% of such isolates were resistant to amikacin, 72% to gentamicin, and 31% to ciprofloxacin. In hospitals in Brooklyn, 78% of ESBL-producing *K. pneumoniae* demonstrated resistance to gentamicin, and 50% to amikacin and to ciprofloxacin (Saurina *et al.*, 2000). This data, as well as the results from other laboratories demonstrate the increasing resistance to non-β-lactam antimicrobials among ESBL producer strains, more and more of which appear to be multi-resistant (Gniadkowski, 2001). Data presented here illustrate this phenomenon very well too. A high percentage (more than 80%) of examined strains were resistant to co-trimoxazole and to aminoglycosides, including amikacin, gentamicin and netilmicin. More than a half of the isolates were also resistant to tetracycline. The only low rate of resistance to fluoroquinolones (2%) may be explained by the fact that these drugs are usually not used in children.

Twenty DDS test positive isolates were further analyzed in the study, by the E-Test ESBL and evaluation of MICs of selected β-lactam antibiotics. These were isolates from the neonatal intensive care unit and the majority of them, mostly *K. pneumoniae* isolates, were selected to the analysis based on their remarkably high-level resistance to cefotaxime and/or ceftazidime. The four *E. coli* isolates were characterized by the relatively weak inhibitor effect in the DDS test. The E-Test ESBL confirmed the ESBL production positive results in the case of all 16 *K. pneumoniae* isolates and gave non-interpretable results for the four *E. coli* isolates. MIC evaluation revealed the typical for ESBL producers pattern of β-lactam MICs in the *K. pneumoniae* isolates, but in the *E. coli* isolates it demonstrated the particularly high MICs of cefotaxime, ceftazidime and aztreonam combinations with clavulanate, which coincided with high cefoxitin MICs (MICs, 128–256 μg ml^{-1}). The MICs of the high inhibitor combinations could not be evaluated with the E-Test and this was the reason of the non-interpretable results obtained with this method. It is very likely that these *E. coli* isolates did not express ESBLs but produced the species-specific AmpC β-lactamase at an elevated level. The DDS test positive result could be false-positive and reflect the weak inhibitory effect of clavulanate on the *E. coli* AmpC enzyme. *E. coli* strains with increased level of AmpC production, although rare, have been observed in different medical institutions (Livermore, 1995; Corvec *et al.*, 2002) (1, 31). The routine clinical microbiology laboratory must have problems with the proper interpretation of such strains, however, classifying them as putative ESBL producers cannot be treated as an error from the most important, clinical point of view.

Further analysis, including molecular typing of the isolates and the identification of the ESBL types is necessary in order to reveal the mechanism of the spread of ESBL producers in the hospital.

Literature

Andrzejewska E., A. Szkaradkiewicz and M. Kaniasty. 1998. Sensitivity to selected β-lactam antibiotics of *Escherichia coli* and *Klebsiella pneumoniae* isolates (in Polish). *Med. Dośw. Mikrobiol.* **50:** 197–205.

Babini G.S. and D.M. Livermore. 2000. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997–1998. *J. Antimicrob. Chemother.* **45:** 183–189.

Bradford P.A. 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol. Rev.* **14:** 933–951.

Bush K. 1996. Is it important to identify extended-spectrum β-lactamase-producing isolates? *Eur.J. Clin. Microbial. Infect. Dis.* **15:** 361–364.

De Champs C., R. Bonnet, D. Sirot, C. Chanal and J. Sirot. 2000. Clinical relevance of *Proteus mirabilis* in hospital patients: a two years survey. *J. Antimicrob. Chemother.* **45:** 537–539.

Cormican M.G., S.A. Marshall and R.N. Jones. 1996. Detection of Exrended-Spectrum β-lactamase (ESBL)-Producing Strains by the E-Test ESBL Screen. *J. Clin. Microbiol.* **34:** 1880–1884.

Corvec S., N. Caroff, E. Espaze, J. Marraillac and A. Reynaud. 2002. –11 mutation in the *ampC* promoter increasing resistance to β-lactams in a clinical *Escherichia coli* strain. *Antimicrob. Agents Chemother.* **46:** 3265–3267.

Drulis-Kawa Z., E. Lewczyk, S. Jankowski and W. Doroszkiewicz. 2000. Infectivity and resistance to antibiotics of uropathogenic Gram-negative rods (in Polish). *Med. Dośw. Microbiol.* **52:**119–127.

ETM, E-Test Technical Manual. 2000. AB BIODISK, Dalvägen 10, S-169 56 Solna, Sweden.

Gniadkowski M. 1997. Beta-lactamases in Gram-negative rods (in Polish). *Nowa Medycyna* **4:** 20–26.

Gniadkowski M. 2001. Evolution and epidemiology of extended-spectrum β-lactamases (ESBLs) and ESBL-producing microorganisms. *Clin. Microbiol. Infect.* **7:** 597–608.

Gniadkowski M., K. Trzciński, A. Pałucha and W. Hryniewicz. 1996. Detection of extended spectrum β-lactamases (ESBL) in clinical isolates of *Klebsiella pneumoniae*: a comparison of the double-disc and the ATB BLSE tests (in Polish). *Diag. Lab.* **32:** 697–709.

Janicka G., D. Wojciechowska, K. Hareńska, J. Porada and C. Kłyszejko. 1997. The resistance to betalactam antibiotics of lactose-positive and lactose-negative strains of *Escherichia coli*. *Acta Microbiol. Polon.* **46:** 339–403.

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

Kędzierska J., M. Doleżal and P. Kachlik. 1999. Resistance patterns of clinical strains of Gram-negative rods in own material (in Polish). *Med. Dośw. Mikrobiol*. **51:** 113–122.

Livermore D.M. 1995. β-lactamases in laboratory and clinical resistance. *Clin. Microbiol. Rev.* **8:** 557–584.

Livermore D.M. and D.F.J. Brown. 2001. Detection of β-lactamase-mediated resistance. *J. Antimicrob. Chemother.* **48:** Suppl. SI, 59–64.

Medeiros A.A. 1997. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. *Clin. Infect. Dis.* **24** (Suppl 1): 19–45.

Meyer K.S., C. Urban, J.A. Eagan, B.J. Berger and J.J. Rahal. 1993. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. *Ann. Inter. Med*. **119:** 353–358.

National Committee for Clinical Laboratory Standards. 2000a. Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard, 7th Edition, M2-A7. NCCLS, Wayne, PA.

National Committee for Clinical Laboratory Standarts. 2000b. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard, 5th Edition, M7-A5. NCCLS, Wayne, PA.

Navarro F. and E. Miró. 2002. Update on CTX-M-type β-lactamases. *Rev. Med. Microbiol.* **13:** 63–73.

Pałucha A., B. Mikiewicz, H. Hryniewicz and M. Gniadkowski. 1999. Concurrent-outbreaks of extended-spectrum β-lactamase-producing organisms of the family Enterobacteriaceae in a Warsaw hospital. *J. Antimicrob. Chemother.* **44:** 489–499.

Paterson D.L., W.-C. Ko, A. von Gottberg, J.M. Casellas, L. Mulazimoglu, K.P. Klugman, R.A. Bonomo, L.B. Rice, J.G. McCormack and V.L. Yu. 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible irganisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. *J. Clin. Microbiol.* **39:** 2206–2212.

Peńa C., M. Pujol, C. Ardanuy, A. Ricart, R. Pallares, J. Linares, J. Ariza and F. Gudiol. 1998. Epidemiology and successful control of a large due to *Klebsiella pneumoniae* producing extended-spectrum β-lactamases. *Antimicrob. Agents Chemother.* **42:** 53–58.

Sanders C.C., A.L. Barry, J.A. Washington, C. Shubert, E.S. Moland, M.M. Traczewski, C. Knapp and R. Mulder. 1996. Detection of extended-spectrum-β-lactamase-producing members of the family *Enterobacteriaceae* with the Vitek ESBL test. *J. Clin.Microbiol.* **34:** 2997–3001.

Saurina G., J.M. Quale, V.M. Manikal, E. Oydna and D. Landman. 2000. Antimicrobial resistance in Enterobacteriaceae in Brooklyn, NY: epidemiology and relation to antibiotic usage patterns. *J. Antimicrob. Chemother.* **45:** 895–898.

Sirot D. 1995. Extended-spectrum plasmid-mediated β-lactamases. *J. Antimicrob. Chemother.* **36:** Suppl. A: 19–34.

Steward C.D., J.K. Rasheed, S.K. Hubert, J.W. Biddle, P.M. Raney, G.J. Anderson, P.P. Williams, K.L. Brittain, A. Oliver, J.E. McGowan and F.C. Tenover. 2001. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum β-lactamase detection methods. *J. Clin. Microbiol*. **39:** 2864–2872.

Szymaniak L., I. Aleksandrowicz and S. Giedrys-Kalemba. 1999. Drug resistance and proticinogenic types of *Proteus mirabilis* strains isolated from urinary tract infections (in Polish). *Med. Dośw. Mikrobiol.* **51:** 323–330.

Ulatowska B., H. Czajkowski, E. Gospodarek and A. Mikucka. 2000. Occurrence of ESBL in *Serratia* species. *Med. Dośw. Mikrobiol.* **52:**17–24.

Wojsyk-Banaszak I. and J. Szczapa. 2000. Central nervous system infections in neonates caused by multiresistant *Klebsiella pneumoniae* (in Polish). *Ginekol. Pol.* **71:** 975–978.