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Prevalence and antimicrobial resistance of extended-spectrum β-lactamases-producing *Escherichia coli* and *Klebsiella pneumoniae* strains isolated in a university hospital in Split, Croatia

Summary. The prevalence of *Escherichia coli* and *Klebsiella pneumoniae* that produce extended-spectrum β-lactamases (ESBL) was investigated in patients of a university hospital in Split, Croatia. Patients were grouped according to age (pediatric vs. adult), antibiotic type, and hospital ward. From Jan. 2001 to Dec. 2002, the susceptibility of *E. coli* and *K. pneumoniae* isolates to antimicrobials was tested. ESBL production was assayed using the double-disk synergy test. ESBL-producing *E. coli* and *K. pneumoniae* were detected in all sites of infection sampled. The percentages of ESBL-positive isolates were higher in the pediatric wards than in the adult wards. The antibiotics most commonly prescribed to patients in all hospital wards belonged to the third-generation cephalosporin group. Among ESBL producers, *E. coli* isolates were more resistant to aminoglycosides, but less resistant to ciprofloxacin and cotrimoxazole. Resistance of *E. coli* and *K. pneumoniae* to ciprofloxacin was exclusively found in isolates from adult patients. None of the isolates, regardless of ESBL production, was resistant to carbapenemes. In addition, the prevalence and antimicrobial resistance of ESBL-producing *E. coli* and *K. pneumoniae* isolates differed between pediatric and adult patients. [**Int Microbiol** 2005; 8(2):119-124]

Key words: *Escherichia coli* · *Klebsiella pneumoniae* · antibiotic resistance · extended-spectrum β-lactamases (ESBL)

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Introduction

Many gram-negative bacilli produce extended-spectrum β-lactamases (ESBL), which are enzymes that mediate resistance to all β-lactams except cephamycins and carbapenems [6,8,21,14]. ESBL-producing bacteria were first isolated in Germany in 1983 [20], but they have since been reported worldwide [6,29]. Most ESBL producers are *Escherichia coli* and *Klebsiella* spp. Infections due to these organisms often occur in outbreaks, and ESBL have therefore become a serious problem in hospitalized patients [5,11,18]. Moreover, since ESBL-producing organisms are frequently also resistant to aminoglycosides, trimethoprim-sulfamethoxazole (cotrimoxazole), and quinolones, the therapeutic choices are limited [6,22,26].

In northern Croatia, the first ESBL-producing *Klebsiella pneumoniae* isolates were reported in hospitals in the capital city, Zagreb, in 1994 [3]. However, there are no published reports concerning the prevalence and resistance of ESBL-producing *Enterobacteriaceae* in southern Croatia. Since *E. coli* and *K. pneumoniae* are the most common producers

of ESBL, we carried out a survey of ESBL-producing clinical isolates of those bacteria in the only hospital in the largest county in southern Croatia. The main objective of the survey was to determine the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* strains isolated as causative agents of infection in patients treated at Split University Hospital over a 2-year period. In addition, the number of ESBL-producing isolates co-resistant to aminoglycosides, cotrimoxazole, and quinolones was determined. Differences in the antimicrobial resistance of ESBL-producing isolates obtained from pediatric and adult patients and from the various hospital wards were analyzed.

Materials and methods

Study design and patients. The study was carried out prospectively from January 1, 2001 to December 31, 2002 at Split University Hospital (SUH), Split, Croatia. SUH is a 1651-bed university teaching hospital and the only hospital in the region. It serves a pediatric and adult population of about 500,000 and acts as a referral hospital for a wider area of southern Croatia, thus covering a population of about one million. For each SUH patient sample, personal data (patient's name, age), type of specimen, and the hospital ward to which the patient had been assigned were obtained. In 2001 and 2002, 50,108 and 50,572 patients, respectively, were admitted to the SUH.

Bacterial isolates. The clinical specimens obtained from inpatients of the SUH yielded 6169 clinical isolates of *Enterobacteriaceae*. Isolates were identified at the species level using standard biochemical tests and microbiological methods. Only one isolate per patient was included in the study.

Antimicrobial susceptibility testing. Disk-diffusion tests were carried out with antibiotic-containing disks (BioRad) on Mueller-Hinton agar plate (BioRad). The results were expressed as susceptible or resistant according to the criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [23]. The following antimicrobial agents were tested: amikacin (30 μg), amoxicillin/clavulanic acid (20/10 μg), ampicillin (10 μg), cefepime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), cotrimoxazole (1.25/23.75 μg), gentamicin (10 μg), imipenem (10 μg), and meropenem (10 μg).

Detection of ESBL production. ESBL production was detected using the double-disk synergy (DDS) test [15]. ESBL presence was assayed using the following antibiotic disks (Becton, Dickinson): cefotaxime (30 μg), cefotaxime/clavulanic acid (30/10 μg), ceftazidime (30 μg), and ceftazidime/clavulanic acid (30/10 μg). *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 and ATCC 35218 strains served as positive controls. For all ESBL-producing isolates of *E. coli* and *K. pneumoniae*, the susceptibility test was reported as resistant to all penicillins, cephalosporins, and aztreonam, irrespective of the individual in vitro test result, as recommended by the NCCLS [24].

Antibiotic consumption. Data on the consumption of the most frequently prescribed antibiotics during the year preceding and during the 2-year study period were obtained from hospital pharmacy records and expressed as defined daily doses (DDD) per 1000 patients per day.

Statistical methods. Differences between proportions were analyzed using the χ^2 test. All differences in which the probability of the null hypothesis was $p < 0.05$ were considered significant.

Results

During the 2-year study period, 6169 consecutive clinical isolates of *Enterobacteriaceae* were isolated. Of these, 3730 (60.5%) were identified as *E. coli* and 715 (11.6%) as *K. pneumoniae*. All isolates of *E. coli* and *K. pneumoniae* were examined for ESBL production. Of the 3730 *E. coli* isolates, 176 (4.7%) were positive for ESBL, while for *K. pneumoniae* 263 of the 715 isolates (36.8%) were ESBL producers. During the entire study period, the prevalence of ESBL-producing *K. pneumoniae* was significantly higher than that of ESBL-producing *E. coli* ($p < 0.001$). There was no significant yearly increase in the prevalence of ESBL-producing *E. coli* or ESBL-producing *K. pneumoniae* during the 2 years studied ($p > 0.05$).

The sources of the ESBL-producing *E. coli* and *K. pneumoniae* isolates tested are shown in Table 1. Respiratory tract infections were the most abundant source of ESBL-producing *E. coli* strains (28.7%; $p < 0.001$), while nearly half of the ESBL-producing *K. pneumoniae* strains were isolated from urinary-tract infections (47.1%; $p < 0.001$).

Table 1. Distribution according to clinical sources of *Escherichia coli* and *Klebsiella pneumoniae* isolates producing extended-spectrum β-lactamases (ESBL)

	Number of ESBL-producing isolates			
	E. coli		*K. pneumoniae*	
Source	Tested	Positive (%)	Tested	Positive (%)
Urinary tract	2879	131 (4.5)	350	165 (47.1)
Respiratory tract	94	27 (28.7)	126	25 (19.8)
Skin, soft tissue	119	8 (6.7)	64	28 (43.8)
Blood	86	4 (4.7)	29	10 (34.5)
Other[a]	552	6 (1.1)	146	35 (24)
Total	3730	176 (4.7)	715	263 (36.8)

[a] Abdominal specimens, pus, cerebrospinal fluid, etc.

The prevalence of ESBL-producing isolates differed between hospital wards and between pediatric and adult patients (Table 2). The prevalence of ESBL-positive *E. coli* and *K. pneumoniae* was highest in isolates obtained from pediatric wards, and differed significantly from the prevalence in neonatal ICU patients. The prevalence of ESBL-positive *E. coli* isolates obtained from adult ICU patients was significantly higher than that in adult patients on other wards (Table 2). By contrast, only one isolate for each of the two bacterial species was cultured from samples taken from neurology-ward patients.

ESBL-positive *E. coli* isolates were more resistant to some aminoglycoside compounds, including amikacin (83.9%) and gentamicin (90.9%), than were ESBL-negative isolates (6.2 and 5.0%, respectively). Although the percentage of ESBL-

Table 2. Frequency of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in the different hospital settings

	Number of ESBL-producing isolates		
	E. coli	*K. pneumoniae*	Total
	Tested/resistant (%)	Tested/resistant (%)	Tested/resistant (%)
Pediatric patients	773/118 (15.3)	187/120 (64.2)	960/238 (24.8
Neonatal ICU	175/49 (28)	68/52 (76.5)	243/101 (41.6)
Pediatric ward	598/69 (11.5)	119/ 68 (57.1)	717/137 (19.1)
	p < 0.0001*	p = 0.013*	p < 0.0001*
Adult patients	2957/58 (1.9)	528/143 (27.1)	3485/201 (5.8)
ICU	39/3 (7.7)	41/12 (29.3)	80/15 (18.7)
Other wardsa	2918/55 (1.9)	487/131 (26.9)	3405/186 (5.5)
	P < 0.0001*	*P* = 0.96	*P* < 0.0001*

[a] Medicine, obstetric/gynecology, neurology, neurosurgery, surgery, urology, orthopedics, oncology, infectious diseases, etc.
* Significant.

positive *E. coli* isolates resistant to ciprofloxacin (1.1%) was fourfold lower than that of ESBL-negative isolates (4.4%), the difference was not significant ($p = 0.07$). In 2001, there were no ESBL-positive *E. coli* isolates resistant to ciprofloxacin, whereas in 2002 two isolates (from the urine of neurosurgery patients) were ciprofloxacin-resistant (resistance in 2002 = 2.3%). ESBL-negative *E. coli* strains were more resistant than ESBL-positive *E. coli* isolates to cotrimoxazole (19.4% vs. 7.1%, $p < 0.0001$). None of the *E. coli* isolates were resistant to carbapenems.

ESBL-positive *K. pneumoniae* isolates were more frequently resistant to amikacin (58.2%), gentamicin (78.4%), ciprofloxacin (10.6%), and cotrimoxazole (32.1%) than were ESBL-negative isolates (6.6%, 8.7%, 4.6% and 13.3%, respectively).

During the 2 years of the study, neither *K. pneumoniae* nor *E. coli* strains resistant to ciprofloxacin were isolated from specimens obtained from neonates or pediatric patients, and only two ciprofloxacin-resistant ESBL-positive *E. coli* isolates were obtained from adult patients (in the neurosurgery ward). However, ciprofloxacin-resistant ESBL-positive *K. pneumoniae* were isolated from adult patients (17.6%). Cotrimoxazole resistance was lower among ESBL-positive *E. coli* isolates obtained from neonates [47/1 (2.1%)] and pediatric patients [66/2 (3.0%) than among isolates from adult patients (57/9 [15.8%]). Of the ESBL-positive *K. pneumoniae* isolates resistant to cotrimoxazole, 18.1% were from neonates, 7.8% from pediatric patients, and 45.3% from adult patients.

Antimicrobial resistance between ESBL-negative and ESBL-positive *E. coli* and *K. pneumoniae* isolates is compared in Table 3. ESBL-negative *K. pneumoniae* isolates were more resistant to gentamicin, whereas ESBL-negative *E. coli* strains were more resistant to cotrimoxazole. Among ESBL-producers, *E. coli* isolates were more resistant to amikacin and gentamicin, whereas *K. pneumoniae* isolates were more resistant to ciprofloxacin and cotrimoxazole.

The most frequently prescribed antibiotics, independent of the year or hospital ward, were third-generation cephalosporins. From 2000 to 2002, their consumption increased from 88 to 157 DDD/1000 patients per day in the pediatric ward, from 4.6 to 12.2 DDD/1000 patients per day, in the neonatology ward, and from 12.0 to 19.0 DDD/1000 patients per day in the neurology department, which, as noted above, had the lowest prevalence of ESBL-producing isolates. Regarding aminoglycosides, the consumption of amikacin by all SUH patients increased from 0.4 to 2.0 DDD/1000 patients per day from 2000 to 2002. By contrast, gentamicin consumption decreased (from 19 to 7 DDD/1000 patients per day). During the same period, there was no consumption of ciprofloxacin or cotrimoxazole by neonatal patients, and ciprofloxacin was not prescribed for pediatric patients. In the neurology department, the ciprofloxacin prescription rate was 0.03 DDD/1000 patients per day. Cotrimoxazole consumption by patients in the pediatric ward, neurology ward, and adult ICU decreased from 76.0, 103.0, and 134.0 DDD/1000 patients per day to 52.0, 78.0, and 127.0 DDD/1000 patients per day, respectively, from 2000 to 2002.

Discussion

The prevalence of ESBL-producing *Enterobacteriaceae* in nosocomial outbreaks of infection has been studied in several countries [18,25,28,32,33,34,38], as well as in multicenter and multinational studies [2,4,17,37]. To our knowledge, the present study is the first survey in southern Croatia focusing on the distribution of ESBL-producing *E. coli* and *K. pneumoniae*.

The overall prevalence of ESBL-producing *E. coli* and *K. pneumoniae* isolates (439/4445, 9.9%) was higher in the SUH in southern Croatia than in hospitals in the Netherlands [34], Italy [33], and Japan [38], but lower than in hospitals in France [1] and Hong Kong [13]. Although, in our study, *E. coli* was more frequently isolated than *K. pneumoniae*, ESBL production was more prevalent in *K. pneumoniae*, which agrees with findings reported in previous studies [10,17,32,33,37]. The prevalence of ESBL-positive *K. pneumoniae* (36.8%) isolates at the SUH was comparable to those in France [7], China [39], and Lebanon [9]. In the 1994 study carried out in Zagreb, in northern Croatia, the prevalence of ESBL-producing *K. pneumoniae* was 19% [3]. In 2000, however, more than 30% of *Klebsiella* strains isolated in Zagreb hospitals were ESBL producers [35]. At the SUH, the prevalence of ESBL producers varied considerably between different wards (Table 2). Our results concerning the high prevalence of ESBL-producing isolates in neonatal and adult ICUs are largely in accordance with published data from other countries [2,12,17,19].

Table 3. Comparison of the antimicrobial resistance of *Escherichia coli* and *Klebsiella pneumoniae* isolates wirh respect to ESBL production

Antibiotic[1]	ESBL-negative			ESBL-positive		
	E. coli (*n* = 3554) T/R (%)[2]	*K. pneumoniae* (*n* = 452) T/R (%)	*P* value	*E. coli* (*n* = 176) T/R (%)	*K. pneumoniae* (*n* = 452) T/R (%)	*P* value
AN	1800/111 (6.2)	408/27 (6.6)	0.82	174/146 (83.9)	220/128 (58.2)	0.0001*
GN	3353/169 (5.0)	414/36 (8.7)	0.001*	176/160 (90.9)	213/167 (78.4)	0.001*
CIP	1844/81 (4.4)	409/19 (4.6)	0.93	169/2 (1.1)	208/22 (10.6)	0.0005*
SXT	2980/577 (19.4)	443/59 (13.3)	0.003*	169/12 (7.1)	212/68 (32.1)	0.0001*
IPM	1700/0 (0)	364/0 (0)		168/0 (0)	227/0 (0)	
MEM	1769/0 (0)	392/0 (0)		171/0 (0)	219/0 (0)	

[1]AN, amikacin; GN, gentamicin; CIP, ciprofloxacin; SXT, cotrimoxazole; IPM, imipenem; MEM, meropenem.
[2] T, number of isolates tested; R, number of isolates resistant; %, percentage of resistant isolates.
* Significant.

Organisms producing ESBL are typically multidrug-resistant [6,9,16,37]. A patient's previous exposure to an antibiotic, especially to extended-spectrum cephalosporins, has been widely reported as a risk factor for infection with ESBL-producing bacteria [30–32]. The high prevalence of ESBL-producing isolates described in this study was probably due to the large amount of third-generation cephalosporins consumed by SUH patients, especially those in the pediatric ward. Although the consumption of third-generation cephalosporins increased throughout the study period, the prevalence of ESBL producers isolated from the SUH remained almost the same, which suggests that ESBL-producing strains are already endemic in the hospital.

Compared with ESBL-negative isolates, ESBL-positive *K. pneumoniae* isolates were more often resistant to aminoglicosydes, ciprofloxacin, and cotrimoxazole. This association between ESBL production and decreased susceptibility to non-β-lactams is in line with the findings of previous investigations [8,32,33]. The lower resistance to ciprofloxacin and cotrimoxazole of ESBL-positive *E. coli* isolates than ESBL-negative isolates (Table 3) is in contrast to the results of other reports [25,27,36], in which higher resistance was usually found in ESBL-positive *E. coli* isolates. The explanation for this difference may be that, in other studies, most ESBL-negative *E. coli* isolates originated from adult patients treated with fluoroquinolones (norfloxacin or ciprofloxacin) or cotrimoxazole for urinary-tract infections. Since most ESBL-producing *E. coli* isolates in our hospital originated from neonates and pediatric patients, the conflicting results possibly reflect the difference in the prescription patterns of cotrimoxazole and ciprofloxacin with respect to patient age.

In our study, the resistance of ESBL-producers to non-β-lactam antibiotics differed considerably between isolates from pediatric and adult patients. Most ESBL-positive strains were resistant to aminoglycosides, in particular to gentamicin. Resistance of *E. coli* and *K. pneumoniae* to ciprofloxacin was found only in isolates obtained from adult patients. These data probably reflect more frequent treatment of adult patients with fluoroquinolones, since a major factor in ciprofloxacin resistance is the consumption of fluoroquinolones [27,28]. Pediatric ESBL-positive isolates were less resistant to cotrimoxazole than isolates from adults, which might be due to prescription patterns, since fluoroquinolones are not administered to children. In contrast to the results of our study, Oteo et al. [25] reported non-significant differences in the resistance of ESBL-positive *E. coli* to fluoroquinolones between isolates from children and those from adults. They also reported higher resistance to cotrimoxazole in children than in adults.

During the entire study period, all ESBL-positive isolates were susceptible to carbapenems, indicating that they are the drugs of choice for treating serious infections caused by ESBL-producing microorganisms [26].

In conclusion, the results of this study also suggest the importance of ESBL-producing *Enterobacteriaceae* as a cause of infections at the SUH. The high prevalence of multidrug-resistant organisms should be taken into account when choosing therapeutic agents, especially for pediatric patients. Since resistance can differ according to geographic location, continuous local monitoring of resistance patterns is necessary to adequately select an empirical antimicrobial therapy. Further studies aimed at unraveling the molecular mechanisms of resistance will provide a better understanding of the epidemiology associated with ESBL-producing species of *Enterobacteriaceae*.

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Prevalencia y resistencia antimicrobiana de cepas de *Escherichia coli* y *Klebsiella pneumoniae* productoras de β-lactamasas de espectro extendido aisladas en un hospital universitario de Split (Croacia)

Resumen. Se ha investigado la frecuencia de *Escherichia coli* y *Klebsiella pneumoniae* productoras de β-lactamasas de espectro extendido (ESBL) en pacientes de un hospital universitario de Split (Croacia). Los pacientes se agruparon en relación con la edad, el tipo de antibiótico recetado y la ubicación en el hospital. Desde enero de 2001 a diciembre de 2002 se realizaron ensayos de susceptibilidad a antimicrobianos en aislados de *E. coli* y *K. pneumoniae*. La producción de ESBL fue ensayada mediante tests de sinergia de disco doble. En todos los lugares de infección se detectaron aislados de *E. coli* y *K. pneumoniae* productores de ESBL. En las salas de pediatría se detectaron los porcentajes más elevados de ESBL-positivos. Los antibióticos más recetados en el hospital fueron las cefalosporinas de tercera generación. Entre los aislados productores de ESBL, los de *E. coli* fueron más resistentes a los aminoglicósidos, pero menos a ciprofloxacina y cotrimoxazol. Sólo se halló resistencia de *E. coli* y *K. pneumoniae* a la ciprofloxacina en aislados obtenidos de pacientes adultos. Ningún aislado, independientemente de la producción de ESBL, fue resistente a los carbapenemos. Se hallaron diferencias en la incidencia y resistencia antimicrobiana de los aislados de *E. coli* y *K. pneumoniae* productores de ESBL entre pacientes pediátricos y pacientes adultos. [**Int Microbiol** 2005; 8(2):119-124]

Palabras clave: *Escherichia coli* · *Klebsiella pneumoniae* · resistencia a antibióticos · β-lactamasas de espectro extendido (ESBL)

Prevalência e resistência antimicrobiana de cepas de *Escherichia coli* e *Klebsiella pneumoniae* produtoras de β-lactamases de largo espetro isoladas em um hospital universitário de Split (Croácia)

Resumo. A freqüência de *Escherichia coli* e *Klebsiella pneumoniae* produtoras de β-lactamasas de amplo espectro (ESBL) foi investigada em pacientes de um hospital universitário de Split (Croácia), em relação com a idade, o grupo de antibiótico receitado e a localização. Desde janeiro de 2001 a dezembro de 2002 foram realizados ensaios de suscetibilidade a diferentes antimicrobianos em isolados de *E. coli* e *K. pneumoniae*. A produção de ESBL foi ensaiada mediante testes de sinergia de disco duplo. Em todos os lugares de infecção foram detectados isolados de *E. coli* e *K. pneumoniae* produtores de ESBL. Nas salas de pediatria foram detectados os valores mais elevados de ESBL. Os antibióticos mais prescritos no hospital foram as cefalosporinas de terceira geração. Entre os isolados produtores de ESBL, os de *E. coli* foram mais resistentes aos aminoglicósidos, mas menos a ciprofloxacina e co-trimoxazol. Só foi achada resistência de *E. coli* e *K. pneumoniae* à ciprofloxacina em isolados obtidos de pacientes adultos. Nenhum isolado, independentemente da produção de ESBL, foi resistente aos carbapenemos. Foram encontradas diferenças na incidência e resistência antimicrobiana dos isolados de *E. coli* e *K. pneumoniae* produtores de ESBL entre pacientes pediátricos e pacientes adultos. [**Int Microbiol** 2005; 8(2):119-124]

Palavras chave: *Escherichia coli* · *Klebsiella pneumoniae* · resistència a antibióticos · β-lactamase de largo espetro (ESBL)