Antimicrobial activity against strains of *Escherichia coli* and *Klebsiella* spp. with resistance phenotypes consistent with an extended-spectrum β-lactamase in Europe

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Extended-spectrum β-lactamases (ESBLs) have continued to evolve after their initial detection in Europe nearly two decades ago. The summary results from the MYSTIC Program (31 medical centers) were utilized to assess the extent of ESBL occurrence in Europe from 1997 to 2000. ESBL phenotype rates in *Klebsiella* spp. (32.8%) and *Escherichia coli* (14.4%) were generally stable, but extensive hospital-to-hospital and unit-to-unit variations were noted. The highest ESBL rates were found in eastern Europe (including Turkey) and in intensive care unit patient populations. Carbapenems remained active against the ESBL-producing strains (meropenem MIC_{90}, 0.25–1 mg/L), while some other agents, such as aminoglycosides, fluoroquinolones, and piperacillin–tazobactam, were significantly less effective. International surveillance initiatives should be maintained to monitor future progression of this important resistance.

Keywords ESBL, MYSTIC, meropenem, *Klebsiella* spp., *E. coli*

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Surveillance studies are valuable tools for assessing the changes in patterns of resistance of clinical isolates to antimicrobial agents [1]. The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program is an international, multicenter, longitudinal surveillance study of antimicrobial activity [2]. The data generated by these surveillance systems are essential for good healthcare resource planning and the use of antimicrobial agents in infection control or therapy, and may avert emerging resistance by predicting antimicrobial agents at risk of selective pressure [3].

The MYSTIC Program began in Europe in 1997 and is expected to continue beyond six years. It is estimated that, by the end of 2002, clinical isolates will have been collected from approximately 120 sentinel hospital units across six continents, all of which prescribe the widely used carbapenem meropenem. Units participating in the MYSTIC Program include intensive care, cystic fibrosis, neutropenia (hematology/oncology) and general wards treating patients with serious infections [2]. The global nature of the MYSTIC Program means that resistance patterns and antimicrobial potency can be compared between different nations as well as between diverse types of center [4].

The trend towards increased antimicrobial resistance shown by many of the Gram-negative bacteria is worrying, and has developed as a consequence of widespread and/or inappropriate use of various agents [5]. Bacterial resistance to β-lactam antimicrobial agents among Gram-negative bacteria occurs principally via β-lactamases, a large number of which have been described in recent years [6]. Strains of primarily *Escherichia coli* and *Klebsiella* spp. are known to harbor the extended-spectrum β-lactamases (ESBLs), resulting in their broad resistance to the 'third-generation' cephalosporins and monobactams [7]. Since 1997, it has been apparent from the MYSTIC Program results that the incidence of plasmid-mediated ESBL-producing isolates is high in some geographic regions or medical centers [8]. These have been generally underestimated in the past, because they have not been routinely tested for. Reduced activity of specific cephalosporins, penicillins, monobactams and β-lactamase inhibitor combinations against the enteric bacilli that

harbor these inactivating enzymes has been shown [8].

This brief report assesses the European data from 1997 to 2000 from the MYSTIC Program in order to examine the diversity, trends and geographic occurrence of ESBL-producing strains in Europe, and to estimate the extent of the problem across the continent.

Data were collected from 31 centers in Europe, located in three MYSTIC Program defined regions: north—Belgium (eight centers), UK (seven centers), Germany (six centers), Sweden (one center); south—Italy (three centers), Switzerland (one center); and east—Turkey (two centers), Czech Republic (one center), Poland (one center), and Russia (one center). The centers sampled consisted of 18 intensive care units (ICUs), four neutropenia units, three cystic fibrosis patient units, and six general hospital wards. For patients from the general wards, isolates predominantly originated from intra-abdominal and/or lower respiratory tract infections. Each center tested 100 Gram-negative isolates. Only the *E. coli* and *Klebsiella* spp. were tabulated, those species having screening criteria published by the National Committee for Clinical Laboratory Standards (NCCLS) [9,10].

The MYSTIC Program broad-spectrum core antimicrobial agents tested (per protocol) were: meropenem, imipenem, cefepime, piperacillin–tazobactam, ciprofloxacin, gentamicin, tobramycin, and amikacin. Tobramycin and amikacin were not routinely tested within the European arm of the MYSTIC Program, but some sites used these agents, so data were considered if they were tested in sufficient numbers. In addition, cefepime was included in the standard set of comparators in the first year, but thereafter, resistance levels were only surveyed by some centers. Minimum inhibitory concentration (MIC) values were determined with agar dilution methods recommended by the NCCLS [9]. Percentage susceptibilities to the antimicrobial agents were determined at the NCCLS susceptibility breakpoint concentrations of $\leq$1 mg/L for ciprofloxacin, $\leq$4 mg/L for meropenem, imipenem, gentamicin and tobramycin, $\leq$8 mg/L for cefepime, and $\leq$16 mg/L for piperacillin–tazobactam and amikacin. Percentage resistance to antimicrobial agents was determined at concentrations of $\geq$4 mg/L for ciprofloxacin, $\geq$8 mg/L for gentamicin and tobramycin, $\geq$16 mg/L for meropenem and imipenem, $\geq$32 mg/L for amikacin and cefepime, and $\geq$128 mg/L for piperacillin–tazobactam [10]. Isolates of *E. coli* and *Klebsiella* spp. were screened for the presence of the ESBL phenotype by NCCLS criteria as defined by a ceftazidime MIC of $\geq$2 mg/L, confirmed by in vitro synergy between ceftazidime and clavulanic acid (4 mg/L); criteria for other species have not been reported [10].

The overall rate of the ESBL phenotype in *E. coli* and *Klebsiella* spp. in Europe was (22.1%). ESBLs were most prevalent among *Klebsiella* spp.

Table 1 Potency and spectrum of antimicrobial agents against ESBL phenotypes and all strains in European regions (1997–2000)

Organism (number tested (ESBL/All))	Antimicrobial agent	MIC_{90} (mg/L)		% resistant[a]	
		ESBL producers	All strains	ESBL producers	All strains
E. coli (189/1310)	Meropenem	0.25	0.12	0.0	0.0
	Imipenem	2	1	1.1	0.4
	Cefepime	8	1	1.8	0.4
	Piperacillin–tazobactam	64	16	5.8	1.8
	Gentamicin	128	8	31.7	8.8
	Tobramycin	128	8	33.1	8.3
	Ciprofloxacin	16	8	23.3	12.7
Klebsiella spp. (306/934)	Meropenem	1	0.12	0.3	0.2
	Imipenem	2	2	0.7	0.2
	Cefepime	32	16	17.8	6.9
	Piperacillin–tazobactam	>128	64	23.9	9.7
	Gentamicin	>128	64	49.7	23.2
	Tobramycin	128	64	60.2	27.7
	Ciprofloxacin	16	4	24.2	10.7

[a]Resistance as defined by NCCLS [10].

Table 2 Activity and potency of antimicrobial agents against ESBL phenotypes in European regions (1997–2000)

Antimicrobial agent	$MIC_{50/90}$ (mg/L)	% S/R[a]	$MIC_{50/90}$ (mg/L)	% S/R	$MIC_{50/90}$ (mg/L)	% S/R	$MIC_{50/90}$	% S/R
E. coli	North (n = 59)[b]		South (n = 64)[c]		East (n = 66)[d]		All (n = 189)	
Meropenem	0.06/0.5	100/0	0.12/0.5	100/0	0.03/0.12	100/0	0.03/0.25	100/0
Imipenem	0.25/2	97/0	0.5/2	100/0	0.12/0.5	97/3	0.25/2	98/1
Cefepime	0.5/8	91/2	2/8	97/0	2/16	87/4	2/8	92/2
Piperacillin–tazobactam	8/64	75/8	4/32	88/0	32/64	47/9	8/64	69/6
Amikacin	2/8	92/0	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]
Gentamicin	1/32	83/15	0.5/32	78/19	32/>128	33/59	2/128	64/32
Tobramycin	2/16	76/12	2/16	86/11	32/>128	30/67	4/128	62/33
Ciprofloxacin	0.12/16	71/29	0.5/32	72/17	0.016/64	73/24	0.25/16	72/23
Klebsiella spp.	North (*n* = 80)[b]		South (*n* = 61)[c]		East (*n* = 165)[d]		All (*n* = 306)	
Meropenem	0.06/0.5	99/0	0.25/1	100/0	0.06/0.25	98/<1	0.06/1	99/<1
Imipenem	0.25/4	98/0	1/2	100/0	0.25/1	98/<1	0.25/2	98/<1
Cefepime	2/8	92/6	16/64	43/38	4/32	77/14	4/32	72/18
Piperacillin–tazobactam	32/>128	46/20	8/32	77/3	64/>128	29/33	32/>128	43/24
Amikacin	8/32	67/4	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]
Gentamicin	2/>128	58/39	4/32	69/25	16/>128	23/64	8/>128	41/50
Tobramycin	2/128	56/40	4/32	51/38	32/>128	19/74	16/128	32/60
Ciprofloxacin	0.25/32	75/21	2/32	49/49	0.12/4	77/16	0.25/16	71/24

[a]Percentage by NCCLS [10] category for susceptible (S) and resistant (R). [b]Four nations: Belgium, Germany, Sweden, and the UK. [c]Two nations: Italy and Switzerland. [d]Four nations: Czech Republic, Poland, Russia, and Turkey. [e]NT, not tested in sufficient numbers (<25.0% of strains detected) to determine spectrum.

($n = 306$, 32.8%), and comparatively low among *E. coli* ($n = 189$, 14.4%). Potency and spectrum data for tested antimicrobial agents against ESBL-producing strains and all strains are shown in Table 1. Potency is expressed as MIC_{90}, and spectrum as the percentage of resistant strains. These data showed high levels of resistance spanning several antimicrobial classes, including cephalosporins, β-lactamase inhibitor combinations, aminoglycosides and fluoroquinolones. ESBL-producing strains were generally more resistant than all strains to the antimicrobial agents tested. ESBL producers were resistant to the other classes of antimicrobial agents, but susceptible to carbapenems (meropenem and imipenem). Gentamicin, tobramycin and ciprofloxacin susceptibilities were particularly compromised in ESBL isolates, especially among *Klebsiella* spp. strains.

ESBL phenotypes were more commonly found in the eastern European countries (231 isolates; 29.9% of the two species tested) than in the northern and southern European regions. Great differences existed between nations, with the majority (57%) of ESBL isolates in eastern Europe coming from Turkish ICU patients. In southern Europe, all ESBL cases emerged in Italy among all four groups of monitored patients, and were especially frequent in ICUs and in neutropenic patients. For the northern European sites, ESBL-producing strains were most prevalent in Germany (36% of all cases), Belgium (30%), and the UK (30%), in ICU clinical settings. Data on susceptibilities and resistances of ESBL phenotypes in *Klebsiella* spp. and *E. coli* by region are shown in Table 2. The MICs for the two carbapenems meropenem and imipenem were low for both the strains of *E. coli* (MIC_{90} 0.25–2 mg/L) and *Klebsiella* spp. (MIC_{90} 1–2 mg/L) that produced ESBLs in all regions of Europe. In contrast, the two aminoglycosides gentamicin and tobramycin showed consistently high resistance rates and MIC values, especially in eastern Europe for *E. coli and* in northern and eastern regions for *Klebsiella* spp. A similar pattern was observed for piperacillin–tazobactam; high MIC_{90} values (*E. coli*, 64 mg/L; *Klebsiella* spp., >128 mg/L) were recorded in northern and eastern Europe for this agent. In contrast, the highest MIC_{90} results shown by cefepime for *Klebsiella* spp. were noted in southern Europe (64 mg/L). The MIC_{50} values for ciprofloxacin were high for *Klebsiella* spp. in southern Europe, and the ciprofloxacin MIC_{90} was in the resistant range in all regions. The highest ciprofloxacin resistance rates in Europe were recorded for *E. coli* (29%) in northern Europe and for *Klebsiella* spp. (49%) in southern Europe. No trend towards greater ESBL occurrence was noted in Europe overall, but individual unit variations were identified (data not shown).

In summary, European data from the MYSTIC Program between 1997 and 2000 showed that ESBLs were more prevalent in *Klebsiella* spp. than in *E. coli.* Geographic differences were observed, with a higher prevalence of ESBL phenotypes in eastern Europe (231 strains), especially Turkey, compared to the remainder of the continent (264 strains), although prevalence was generally high throughout Europe. Meropenem and imipenem both exhibited excellent activity against *E. coli* and *Klebsiella* spp., and, of the range of antimicrobial agents tested, were shown to have the highest susceptibility rates. This study therefore showed that the carbapenems are valuable options in the treatment of infections caused by Enterobacteriaceae, even those involving ESBL-producing organisms. The prevalence of ESBLs presented here, especially in *Klebsiella* spp., agrees with previously reported observations in Europe [11,12], and is high enough to warrant consideration when selecting initial therapy if both the organism and its susceptibility are unknown and where ESBL-producing phenotypes have been encountered.

Control of these problematic ESBL strains of *E. coli* and *Klebsiella* spp. in Europe will require comprehensive programs of infection control and formulary management, supplemented by quality surveillance systems at the local level and by global systems such as the MYSTIC Program.

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