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Emergence of *Enterobacteriaceae* Isolates Producing CTX-M Extended-Spectrum β-Lactamase in Austria

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Among 149 extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* isolates collected from patients in southeast Austria from 1998 to 2004, 38 *Escherichia coli* isolates and 11 *Klebsiella* spp. were CTX-M producers. The proportion of CTX-M-producers among all ESBL producers rose from 0% in 1998 to 58% in 2004. In general, CTX-M-producers had heterogeneous pulsed-field gel electrophoresis patterns, but one *E. coli* isolate was identical to a United Kingdom epidemic CTX-M-15-producing strain, although no epidemiological link with the United Kingdom was apparent.

In the past 10 years, CTX-M-type extended-spectrum β-lactamases (ESBLs) have increased dramatically in prevalence among clinical isolates of *Enterobacteriaceae* in Europe and Asia, having spread previously in South America (2). In contrast to most TEM and SHV ESBLs, CTX-M β-lactamases preferentially hydrolyze cefotaxime over ceftazidime (11). They are classified into five phylogenetic groups, most or all of which evolved from the chromosomal β-lactamases of *Kluyvera* spp. (2, 7, 9).

At present there are no published data about the genetic background of ESBL-producing *Enterobacteriaceae* in southeast Austria. The aim of the present study was therefore to investigate the prevalence of CTX-M producers among ESBL-producing clinical isolates of *Enterobacteriaceae* in Graz, southeast Austria.

We analyzed the first bacterial isolates from 149 patients with infections due to ESBL-producing *Enterobacteriaceae* collected in the routine microbiology laboratory of the Medical University of Graz between January 1998 and December 2004. All bacterial isolates were independent. The isolates were retested for species identification and antibiotic susceptibilities with a VITEK 2 system (bioMérieux) using the ID-GN and the AST-N020 test cards, and the results were interpreted using the criteria recommended by the CLSI (formerly NCCLS) (6). All isolates were screened for ESBL production by the Etest ESBL screen method using strips with cefotaxime, ceftazidime, and cefepime (AB Biodisk, Solna, Sweden). The producers collected included 89 *Klebsiella* spp. (71 *Klebsiella pneumoniae* and 18 *Klebsiella oxytoca* isolates) and 60 *Escherichia coli* isolates; 117 were from hospitalized patients, while 32 were from patients attending general practices in southeast Austria. ESBL-producing isolates were from the genitourinary tract ($n = 58$), upper respiratory tract ($n = 38$), fecal screens ($n = 21$), wounds ($n = 8$), or unknown sources ($n = 24$).

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For detection and differentiation of $bla_{\text{CTX-M}}$ alleles, all isolates with an ESBL phenotype were screened by multiplex PCR using nine primers specific for alleles encoding CTX-M enzymes belonging to phylogenetic groups 1 (CTX-M-1-related enzymes), 2 (CTX-M-2-related enzymes), and 9 (CTX-M-9 related enzymes) as well as for $bla_{\text{CTX-M-8}}$ and $bla_{\text{CTX-M-25/-26}}$ (13). Additionally, a 400-bp fragment spanning the link between an IS*26* element and $bla_{\text{CTX-M-15}}$ was sought by PCR to identify members of a CTX-M-15-producing strain (designated strain A) isolated in many United Kingdom laboratories (12).

All isolates producing CTX-M enzymes were compared by pulsed-field gel electrophoresis (PFGE) of XbaI-digested genomic DNA using a CHEF DRII apparatus (Bio-Rad Laboratories, Hemel Hempstead, United Kingdom), as described recently (5, 12). Banding patterns were analyzed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) and interpreted using the guidelines suggested by Tenover et al. (10). *E. coli* isolate 499 (12), a CTX-M-15 producer belonging to United Kingdom epidemic strain A, was included as a comparator.

Forty-nine of 149 (33%) isolates produced CTX-M enzymes belonging to group 1 or 9 (Table 1). The majority of the isolates with $bla_{\text{CTX-M}}$ genes were found in 2003 and 2004, by which time 43 to 58% of all ESBL producers had CTX-M ESBLs, compared with 0 to 11% in 1998 to 1999. The majority of CTX-M producers (38/49; 78%) had group 1 enzymes; the remainder had group 9 enzymes. Most (63%) CTX-M producers were recovered from patients with urinary tract infections. In total, more than 70% of all ESBL-producing isolates of community origin screened for the presence of CTX-M enzymes were found to be positive, compared with 21% of all ESBL producers of hospital origin. However, we did not regard either previous treatment with antibiotics or previous hospitalization of the patients attending general practices in southeast Austria. When compared by PFGE, all the 11 klebsiellae with $bla_{\text{CTX-M}}$ genes were found to be unrelated to one another, whereas the 38 *E. coli* isolates with $bla_{\text{CTX-M}}$ genes included 7 pairs of isolates with indistinguishable or closely related restriction patterns. Two identical isolates were isolated in the same hospital department over 15 months, and one

TABLE 1. Numbers of ESBL-producing *E. coli* and *Klebsiella* sp. isolates

Yr of isolation	*E. coli*					*Klebsiella* spp.				
	No. of clinical isolates	No. (%) of ESBL producers	CTX-M group 1/ group 9	% of CTX-M enzymes among ESBLs	% of non-CTX-M enzymes among ESBLs	No. of clinical isolates	No. (%) of ESBL producers	CTX-M group 1/ group 9	% of CTX-M enzymes among ESBLs	% of non-CTX-M enzymes among ESBLs
1998	5,004	3 (0.06)	0/0	0	100	1,059	16 (1.5)	0/0	0	100
1999	5,300	2 (0.04)	0/1	50	50	1,140	12 (1)	1/0	8	92
2000	4,636	6 (0.13)	2/0	33.3	66.6	938	11 (1.2)	0/0	0	100
2001	6,382	3 (0.05)	1/0	33.3	66.6	1,114	15 (1.3)	0/0	0	100
2002	6,685	7 (0.1)	4/0	57.1	42.9	1,129	7 (0.6)	2/0	28.6	70.4
2003	7,004	19 (0.3)	11/2	68.4	31.6	1,163	8 (0.7)	0/1	12.5	87.5
2004	7,259	20 (0.3)	12/5	85	15	1,209	20 (1.6)	5/1	30	70
Total	42,270	60	30/8	36.6	63.3	7,752	89	8/3	12.4	87.6

E. coli isolate, recovered from the genitourinary tract of a 48-year-old woman, was indistinguishable from the CTX-M-15-producing *E. coli* strain 499, which belongs to United Kingdom epidemic strain A. The presence of an IS*26* element within the terminal inverted repeat of the IS*Ecp1*-like element and upstream of $bla_{\text{CTX-M}}$ was shown to be a useful and characteristic molecular marker for isolates belonging to epidemic strain A in the United Kingdom (12) and was confirmed by PCR for this Austrian isolate. The woman had been suffering from a urinary tract infection and colpitis, which became manifest when she was on vacation in southern Italy. She had not been to the United Kingdom, and she had no known contact with anyone who had been to the United Kingdom.

All the *Klebsiella* and *E. coli* isolates with $bla_{\text{CTX-M}}$ genes were susceptible to meropenem (MIC, ≤0.25 mg/liter). Rates of susceptibility of $bla_{\text{CTX-M}}$-positive klebsiellae and *E. coli* to other agents were, respectively, 18% and 100% for amikacin, 36% and 63% for gentamicin, 55% and 66% for cotrimoxazole, 73% and 45% for ciprofloxacin, and 54% and 92% for piperacillin-tazobactam.

In recent years, CTX-M β-lactamases have spread among *Enterobacteriaceae* in most parts of the world (2). In this study describing the evolution of CTX-M enzymes among clinical isolates of *E. coli* and *Klebsiella* spp. in southeast Austria, the rate of their dissemination increased from 0% in 1998 to 58% in 2004, whereas the prevalence of other, non-CTX-M enzymes remained constant during the same period. Nosocomial outbreaks with producers of CTX-M-type ESBLs have been reported in Poland, the Far East, and South America (1, 2, 4), while the emergence of ESBL-producing *Enterobacteriaceae* in the community has been reported in Spain, Israel, and the United Kingdom (2, 12). In the present study, the CTX-M ESBLs were significantly associated with patients attending community practices ($P = 0.0148$ by the chi-square test). All of the community isolates with $bla_{\text{CTX-M}}$ genes originated from patients suffering from genitourinary tract infections.

The PFGE typing data proved that most infections with CTX-M β-lactamase-producing *Enterobacteriaceae* in southeast Austria were sporadic. Interestingly, the PFGE type of one *E. coli* isolate was indistinguishable from that of a United Kingdom epidemic CTX-M-15-producing strain, but no epidemiological link with the United Kingdom was apparent; rather, the patient had developed the urinary tract infection in southern Italy. CTX-M enzymes have been reported in *E. coli* isolates from hospitalized patients from Italy, as well as from cats and dogs in Rome (3, 8). However, those strains were not compared with United Kingdom strain A for epidemiologic analysis.

As elsewhere, CTX-M enzyme production in these *E. coli* and *Klebsiella* sp. isolates was often associated with resistance to other antibiotics, such as ciprofloxacin, cotrimoxazole, and aminoglycosides, but no carbapenem-resistant isolates were detected. A high proportion of the CTX-M-producing isolates also remained susceptible to piperacillin-tazobactam; this is in contrast to United Kingdom isolates, most of which also have an OXA-1 β-lactamase, conferring resistance to inhibitor combinations.

In conclusion, CTX-M β-lactamase-producing strains of *Enterobacteriaceae* are an emerging problem in Austria, as elsewhere in Europe, and this is a matter of public health importance. The finding of a "United Kingdom" epidemic CTX-M-15 β-lactamase-producing *E. coli* isolate in Austria is intriguing, and the international dissemination of this strain requires further investigation.

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