Extended-Spectrum β-Lactamases of the CTX-M Type Now in Switzerland[▽]

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The epidemiology of clavulanic acid-inhibited extended-spectrum β-lactamases (ESBLs) was investigated among infection-associated enterobacterial isolates at the University Hospital in Lausanne, Switzerland, from January 2004 to June 2005. Out of 57 nonrepetitive ESBL producers (prevalence rate of 0.7%), 45 produced CTX-M-like ESBLs. CTX-M enzymes were mostly from clonally nonrelated *Escherichia coli* isolates, from urinary infections and community-acquired infections. Pediatric patients (20 out of 57) accounted for a large number of CTX-M producers. CTX-M-15 was the most frequent CTX-M-type enzyme. The plasmid-located *bla*$_{CTX-M}$ genes were associated with either IS*Ecp1* or IS*CR1* insertion sequences. This study is the first published report of CTX-M-type β-lactamases in Switzerland.

Plasmid-mediated extended-spectrum β-lactamases (ESBLs) were first identified in a *Klebsiella pneumoniae* isolate in Germany in 1983 (14). Since then, ESBL-positive *Enterobacteriaceae* have been isolated worldwide mostly from hospitalized patients (18). These enzymes hydrolyze significantly expanded-spectrum cephalosporins such as cefotaxime and ceftazidime and the monobactam aztreonam, sparing carbapenems. Their activity is inhibited in vitro by clavulanic acid. Until the 2000s, most of the ESBLs were structurally related to the narrow-spectrum TEM- and SHV-type β-lactamases, with one to several amino acid substitutions surrounding their active site (18). Beginning in the late 1990s, novel types of ESBLs, the CTX-M enzymes, emerged worldwide, mostly from *Escherichia coli*. The CTX-Ms are mostly from community-acquired isolates (2, 22). The over 50 CTX-M enzymes so far reported may be grouped into five main subgroups according to amino acid sequence identity (CTX-M-1, -M-2, -M-8, -M-9, and -M-25) (2). Most of the CTX-Ms hydrolyze cefotaxime better than ceftazidime. However, several CTX-Ms including CTX-M-15 (2, 12, 25), which is now the most widespread CTX-M enzyme worldwide (6), hydrolyze ceftazidime efficiently (25).

Since those CTX-Ms are reported increasingly in France (8, 15, 17, 26), Italy (4, 20), and recently Austria (9), it was interesting to search for those enzymes in Switzerland, a country known to have a strict policy of antibiotic prescription and to have an overall low level of multidrug resistance in bacteria (10).

The aim of the present study was to estimate the prevalence and the type of the ESBLs produced by enterobacterial isolates among nonrepetitive clinical isolates over an 18-month period from January 2004 to June 2005 at the University Hospital of Lausanne, Switzerland.

MATERIALS AND METHODS

Bacterial isolates. ESBL-producing enterobacterial isolates resulted from the screening of 8,259 enterobacterial isolates obtained from infection samples that were sent to the Department of Microbiology of the Lausanne University Hospital, Switzerland, from January 2004 to June 2005. Isolates were first identified by using the Vitek2 system (bioMérieux SA, Marcy-l'Etoile, France). Electrocompetent *E. coli* TOP10 (Invitrogen, Cergy Pontoise, France) was used as a recipient strain in transformation experiments. *E. coli* NCTC 50192, harboring 154-, 66-, 38-, and 7-kb plasmids, was used as a plasmid-containing reference strain (27).

Susceptibility testing and screening for ESBL-producing isolates. The antibiotic susceptibility of enterobacterial clinical isolates was determined with the Vitek2 Advance Expert System (bioMerieux) and by the disk diffusion method on Mueller-Hinton (MH) agar plates with β-lactam and non-β-lactam antibiotic-containing disks (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France), according to the Clinical and Laboratory Standards Institute guidelines (7). The double-disk synergy test was performed with ceftriaxone, ceftazidime, aztreonam, cefpodoxime, and amoxicillin-clavulanic acid disks using different disk spacings (20, 25, 30, and 40 mm) on MH agar plates, and the results were interpreted as described previously (11). The Etest strips containing cefepime plus clavulanic acid (AB Biodisk, Solna, Sweden) were also used. Then, MICs were determined for selected β-lactams by an agar dilution technique on MH agar with an inoculum of 10^4 CFU per spot, as described previously (23). MICs of several β-lactams were determined alone or in combination with a fixed concentration of either clavulanic acid (4 μg/ml) or tazobactam (4 μg/ml). MICs were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (7).

PCR amplification for detection of ESBL genes, analysis of their genetic environment, and sequencing. Under standard PCR conditions (27), a series of primers was used for detection of several Ambler class A β-lactamase genes (Table 1). Detection was performed for genes encoding TEM (PRETEM-1 and PRETEM-2), SHV (OS5 and OS6), and CTX-M (CTX-MA1 and CTX-MA2) (8, 25). For each reaction, 0.5 μg of whole-cell DNA of the ESBL-possessing enterobacterial isolates or 0.5 μg of plasmid DNA from *E. coli* TOP10 electroporants was used.

The genetic environment of the *bla*$_{CTX-M}$ genes was characterized by PCR (16) since different genetic elements are associated with the *bla*$_{CTX-M}$ genes such as IS*Ecp1*-like insertion sequences and the IS*CR1* element comprising *orf513* which is embedded in a *sul1*-type integron (6). Whole-cell DNA of the isolates was extracted as described previously (12). The regions located upstream of the

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TABLE 1. Sequences of primers used for detection of bla_{CTX-M} genes and their genetic environment

Primer name	Primer sequence	Location
CTX-MA1	5′-SCSATGTGCAGYACCAGTAA-3′	bla_{CTX-M} gene
CTX-MA2	5′-CCG CRA TAT GRT TGG TGG TG-3′	bla_{CTX-M} gene, reverse primer
CTXM2A	5′-GCC GCT CAA TGT TAA CGG-3′	bla_{CTX-M} gene (CTX-M-2 group)
CTXM2B	5′-GAA ACC GTG GGT TAC GAT-3′	bla_{CTX-M} gene (CTX-M-2 group), reverse primer
CTXM9A	5′-CTG ATG ATG TAA CAC GGA TTG AC-3′	bla_{CTX-M} gene (CTX-M-9 group)
CTXM9C rev	5′-AGC GCC CCA TTA TTG AGA G-3′	bla_{CTX-M} gene (CTX-M-9 group), reverse primer
TOHO2b rev	5′-TTA CAG CCC TTC GGC GAT-3′	bla_{CTX-M} gene (CTX-M-9 group), reverse primer
CTXMpréB	5′-CAC TTT GCC GTC GTC TAA GGC G-3′	bla_{CTX-M} gene (CTX-M-1 group), reverse primer
ISEcpPROM+	5′-TGC TCT GTG GAT AAC TTG C-3′	IS$Ecp1$ upstream of the promoter
ISEcpPROM−	5′-GCA GTC TAA ATT CTT CGT G-3′	IS$Ecp1$ downstream of the promoter
IS903Bint	5′-GCT TTT TGA CTT TCC ACT CGC-3′	IS903B transposase, reverse primer
Orf513-D3	5′-CTC ACG CCC TGG CAA GGT TT-3′	Orf513
Orf513-D5	5′-CTT TTG CCC TAG CTG CGG T-3′	Orf513, reverse primer
Orf513-5′ext	5′-CAG CTG GTA GAG CAG CGT C-3′	5′ end of Orf513, reverse primer
OS5	5′-TTA TCT CCC TGT TAG CCA CC-3′	bla_{SHV} gene
OS6	5′-GAT TTG CTG ATT TCG CCG G-3′	bla_{SHV} gene, reverse primer
PRETEM-1	5′-GTA TCC GCT CAT GAG ACA ATA-3′	bla_{TEM} gene
PRETEM-2	5′-TCT AAA GTA TAT ATG AGT AAA CTT GGT CTG-3′	bla_{TEM} gene, reverse primer

bla_{CTX-M} genes were amplified with primers annealing to IS$Ecp1$ and to $orf513$ of IS$CR1$ together with primers for the bla_{CTX-M} genes (26). The sequences located downstream of the bla_{CTX-M} genes were studied by PCR experiments with the forward primer CTX-MA1 and the reverse primer CTXMpreB or IS903Bint. When the $orf513$ gene was found upstream of bla_{CTX-M}, a PCR experiment was performed with primer CTX-MA1 and reverse primer qacED-1B in order to search for a $sul1$-type integron structure as previously described with a duplication (even partial) of the IS$CR1$ element (16, 26).

For direct DNA sequencing, PCR products were purified using PCR purification columns (QIAGEN, Courtaboeuf, France). The sizes of the sequenced bla_{SHV}, bla_{TEM}, and bla_{CTX-M} genes were 795, 861, and 876 bp, respectively. Sequencing reactions were performed using specific primers and an automated sequencer (ABI 377; Applied Biosystems, Foster City, CA). The nucleotide and deduced protein sequences were analyzed with software available over the Internet at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

Plasmid transfer and analysis. Plasmid DNAs of enterobacterial isolates were extracted using the Kieser technique (13). They were then electroporated into *E. coli* TOP10, and recombinant strains were selected onto cefotaxime-containing (1 μg/ml) Trypticase soy agar plates. Plasmid DNAs of these transformants were detected by electrophoresis on a 0.7% agarose gel.

Hybridization. DNA-DNA hybridizations were performed as described previously with a Southern transfer of an agarose gel containing plasmid DNA from *E. coli* TOP10 electroporants (25). The probes consisted of a ca.-500-bp PCR fragment generated from isolate 23 producing CTX-M-15, isolate 8 producing CTX-M-14, and isolate 25 producing CTX-M-2. They were internal to the bla_{CTX-M} genes (15). Labeling of the probe and signal detection were carried out using a nonradioactive labeling and detection kit according to the manufacturer's instructions (Amersham Pharmacia Biotech).

PFGE. Whole-cell DNAs embedded in 1% agarose plugs (Bio-Rad) were digested with XbaI restriction enzyme (Amersham Pharmacia Biotech) and separated in a 1% pulsed-field certified agarose gel (Bio-Rad) by using a CHEF DRII system (Bio-Rad), as described previously (24). Pulsed-field gel electrophoresis (PFGE) was performed at 14°C, with a 6-V/cm current, a switch angle of 120°, and for *E. coli*, a run time of 12 h followed by a run time of 12 h, with two linear switch ramps of 4 and 12 s and 15 to 36 s, and for *K. pneumoniae*, switch times of 2 to 32 s for 20 h. After migration, gels were stained in an 0.5-mg/ml ethidium bromide solution, and PFGE results were analyzed according to the criteria of Tenover et al. (28).

RESULTS

Epidemiology and PCR detection of β-lactamase genes. A total of 57 nonrepetitive ESBL-positive isolates were collected from 54 patients. The prevalence of ESBL producers was 0.7%. Any enterobacterial isolate flagged as a possible ESBL producer by the Vitek2 Advance Expert System was tested by the double-disk test and by the Etest strip containing cefepime/cefepime plus clavulanic acid. The isolates were *E. coli* ($n = 31$), *Klebsiella pneumoniae* ($n = 17$), *Enterobacter cloacae* ($n = 6$), *Proteus mirabilis* ($n = 2$), and *Klebsiella oxytoca* ($n = 1$). The sex ratio of patients was 32/22 (female/male). The isolates were from hospitalized patients (43/54), outpatients (11/54), and significantly from pediatric patients (19/54). Out of the 43 hospitalized patients, nine isolates were obtained within 48 h of their hospitalization. Therefore, community-acquired isolates were from 20 patients (nine hospitalized patients plus 11 outpatients).

Three patients had two different ESBL-positive isolates (*E. coli* and *K. pneumoniae* in two cases and *E. coli* and *P. mirabilis* in one case) that were collected at different times. Moreover, the same ESBL-positive isolate was identified from a urinary sample from a woman and from the eyes of her twins.

The ESBL-positive isolates were mostly from urine (63%) and from pus (24%) but also from respiratory tract or blood. Most of the patients were treated successfully with a carbapenem-containing antibiotic regimen (data not shown).

PCR experiments with primers specific for the bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes yielded bla_{CTX-M}-positive results for 45 out of the 57 isolates (Table 2). A bla_{CTX-M} gene was identified in 14 out of the 20 community-acquired isolates. The bla_{TEM} and bla_{SHV} genes encoding ESBLs were identified in all the 12 bla_{CTX-M}-negative isolates. A single isolate (*E. coli* 14) expressed two different ESBLs.

Molecular identification of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes and genetic environment of bla_{CTX-M} genes. Seventy-nine percent of ESBLs were CTX-M enzymes (46/57) (Table 2). Most of them (78%, 36/46) belonged to the CTX-M-1 group, CTX-M-15 being predominant (34/36). Twenty percent of the CTX-Ms were CTX-M-9-like (9/46), being either CTX-M-14 or CTX-M-9. A single isolate produced CTX-M-2. A single *E. coli* isolate produced both CTX-M-14 and CTX-M-15. Sixty percent of the CTX-M producer isolates produced also the narrow-spectrum β-lactamase TEM-1.

E. coli was the main ESBL producer. It was identified for 63% of the CTX-M-positive isolates, and CTX-M enzymes

TABLE 2. Characterization of acquired β-lactamases in ESBL-positive isolates

Isolate	β-Lactamase type(s)[a]:		
	CTX-M	TEM	SHV
E. cloacae			
1	**CTX-M-9**		
2		TEM-1	**SHV-5**
3			**SHV-5**
4			**SHV 5**
5		TEM-1	**SHV-5**
6		TEM-1	**SHV-5**
E. coli			
7	**CTX-M-15**	TEM-1	
8	**CTX-M-14**	TEM-1	
9	**CTX-M-15**		
10	**CTX-M-14**	TEM-1	
11	**CTX-M-15**		
12	**CTX-M-14**		
13	**CTX-M-1**		
14	**CTX-M-15, CTX-M-14**	TEM-1	
15	**CTX-M-15**		
16	**CTX-M-15**	TEM-1	
17	**CTX-M-15**	TEM-1	
18	**CTX-M-1**	TEM-1	
19		**TEM-53**	
20	**CTX-M-15**	TEM-1	
21	**CTX-M-14**	TEM-1	
22	**CTX-M-15**		
23	**CTX-M-15**	TEM-1	
24		TEM-1	**SHV-5**
25	CTX-M-2		
26	**CTX-M-15**		
27	**CTX-M-15**	TEM-1	
28	**CTX-M-15**	TEM-1	
29	**CTX-M-15**	TEM-1	
30	**CTX-M-14**		
31		**TEM-52**	
32	**CTX-M-15**		
33	**CTX-M-15**		
34	**CTX-M-15**		
35	**CTX-M-15**	TEM-1	
36	**CTX-M-15**		
37	**CTX-M-15**		
K. oxytoca			
38	**CTX-M-15**	TEM-1	
K. pneumoniae			
39	**CTX-M-15**	TEM-1	
40	**CTX-M-9**		
41		TEM-1	**SHV-5**
42		TEM-1/**TEM-4**	SHV-11
43			**SHV-5**
44	**CTX-M-15**	TEM-1	
45			**SHV-2a**
46	**CTX-M-15**		
47	**CTX-M-15**	TEM-1	
48	**CTX-M-15**		
49	**CTX-M-15**		
50	**CTX-M-15**	TEM-1	
51	**CTX-M-15**	TEM-1	
52	**CTX-M-15**	TEM-1	
53	**CTX-M-15**		
54	**CTX-M-15**	TEM-1	
55	**CTX-M-15**	TEM-1	
P. mirabilis			
56	**CTX-M-15**	TEM-1	
57	**CTX-M-14**	TEM-1	

[a] The ESBL names are in bold.

accounted for 91% of the ESBLs in that species. The prevalence of CTX-M among ESBL types was also high in *K. pneumoniae* (76%). Only one out of the six *E. cloacae* isolates produced a CTX-M enzyme. The other ESBLs were mostly of the SHV type in *E. cloacae* and *K. pneumoniae* (Table 2).

The genetic structure surrounding the bla_{CTX-M} genes was then determined. IS*Ecp1* was identified upstream of 42 out of the 46 bla_{CTX-M} genes (91%). The right boundary of IS*Ecp1* was located between 43 and 80 bp upstream of the start codon of bla_{CTX-M} genes. IS*Ecp1* was identified at 80 bp upstream of the $bla_{CTX-M-1}$ gene and at 48 bp upstream of $bla_{CTX-M-15}$ genes. A 43-bp region with an identical sequence was found between IS*Ecp1* and the start codon of $bla_{CTX-M-9}$-like genes ($bla_{CTX-M-9}$ and $bla_{CTX-M-14}$). Downstream of four out of six $bla_{CTX-M-14}$ genes (14, 12, 8, and 21), an IS*903*-like element was found as already described for this gene (24). IS*Ecp1* was not identified upstream of the $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$ genes, as previously reported (21). IS*CR1* was found in isolates harboring $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$ genes (Fig. 1).

Antibiotic susceptibility results. The isolates were resistant to amino-, ureido-, and carboxypenicillins, and clavulanic acid and tazobactam addition partially restored the activity of those antibiotics (Table 3). Isolates producing CTX-Ms were resistant to cefotaxime whereas this was not always the case for TEM and SHV producers. The CTX-M-15 producers were resistant also to ceftazidime except for two isolates (*E. coli* 37 and *P. mirabilis* 56). Susceptibilities to cefepime and cefpirome varied whereas susceptibilities to carbapenems were constant. Disk diffusion susceptibility testing indicated that overall resistance rates of ESBL producers were 2, 63, 50, 75, and 30% for amikacin, sulfonamides, ciprofloxacin, tetracyclines, and chloramphenicol, respectively, with no significant difference between CTX-M and non-CTX-M producers (data not shown).

PFGE. Analysis of XbaI-restricted DNA of CTX-M-producing *E. coli* and *K. pneumoniae* isolates showed that the bla_{CTX-M}-positive isolates were mostly nonclonally related. Only three *E. coli* isolates (isolates 11, 15, and 36) were clonally related or identical. Those isolates produced CTX-M-15, and two of them (isolates 11 and 15) were identified from patients hospitalized in the same ward. Analysis of the PFGE profiles of CTX-M-15-producing *K. pneumoniae* isolates showed that three isolates (50, 51, and 52) were undistinguishable. They were from urine from a mother of premature twins and from conjunctivitis of her twins.

Plasmid analysis and hybridization. Plasmid DNAs of the CTX-M-producing isolates were extracted and analyzed by gel electrophoresis. Their sizes ranged from 7 to 170 kb with one to five plasmids per isolate. The plasmid DNAs hybridized with any of the three internal probes for $bla_{CTX-M-1}$-like, $bla_{CTX-M-2}$, and $bla_{CTX-M-9}$-like (data not shown). A single hybridization signal was evidenced in each isolate, except for *E. coli* isolate 14, which exhibited two hybridization signals. These results showed that all CTX-M producers except one contained a single bla_{CTX-M} gene. Those β-lactamase genes were located on 50- and 170-kb plasmids. Four out of the seven $bla_{CTX-M-14}$ genes were located on similarly sized plasmids (ca. 80 kb) although the CTX-M producers were not clonally related. The $bla_{CTX-M-15}$ genes were located mostly on plasmids varying in size. The $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$ genes of *E. coli* isolate 14 were located on two different plasmids of 70 kb and 155 kb,
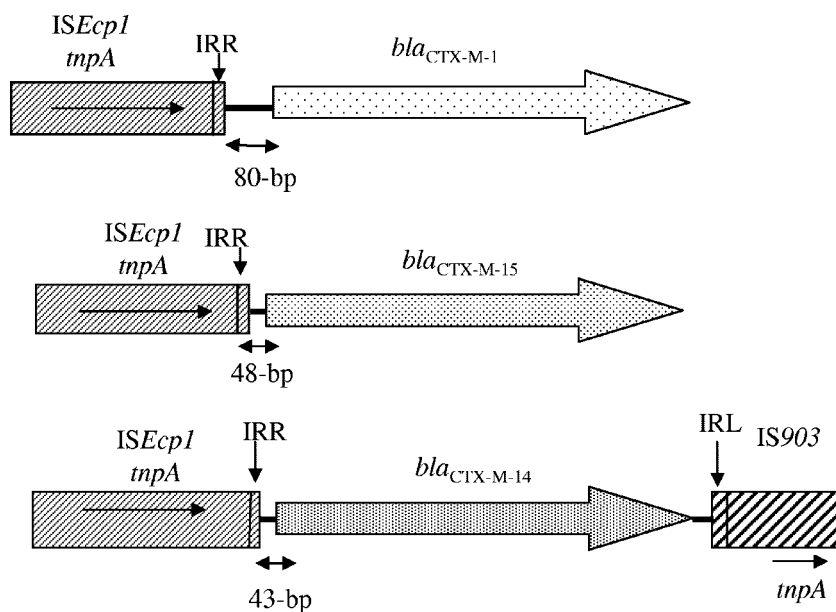
FIG. 1. Surrounding DNA sequences for the bla_{CTX-M} genes for the 42 isolates. Row 1, *E. coli* 13 and 18; row 2, *E. coli* 7, 9, 11, 14, 15, 16, 17, 20, 22, 23, 26, 28, 29, 32, 33, 34, 35, 36, and 37; *K. oxytoca* 38; *K. pneumoniae* 39, 44, 46, 47, 48, 49, 50, 51, 52, 53, 54, and 55; *P. mirabilis* 56 and 57; row 3, *E. coli* 8, 10, 12, 14, 21, and 30 and *P. mirabilis* 57. IRR and IRL, inverted repeat (right) and inverted repeat (left), respectively.

respectively. These results indicated that the spread of bla_{CTX-M} genes resulted from both clonal and plasmid spread.

DISCUSSION

The results of this work provided insights into the molecular epidemiology of the spread of ESBLs in enterobacterial isolates responsible for infections at a university hospital in Switzerland. The prevalence rate of ESBL producers was 0.7%, which is very similar to that reported in the nearby country Austria in 2004 (9). However, this rate is lower than that reported in The Netherlands (7.8% in Amsterdam, 2004 [1]), in France (2.3% at Bicêtre Hospital, 2004 [P. Nordmann, personal data]), Italy (1.6%, 2003 [4]), and the United States

(4.9%, 2001 to 2002 [19]). Variable prevalence rates of ESBL producers may be related in part to differences in antibiotic policy (more quinolone used than β-lactams for treating community patients in Switzerland [10]) but also to differences in urban sizes (Paris area or Amsterdam versus Lausanne, for example). The bla_{CTX-M} genes were widespread among ESBL producers, 79%, which was a slightly higher value than that reported at Bicêtre Hospital (65%) (personal data), in Amsterdam (64%) (1), and in Austria (58%) (9). A recent study performed in several Swiss laboratories between 2001 and 2003 identified 63.4% of ESBL producers as producing CTX-Ms (5).

ESBLs of *E. coli* were mostly CTX-Ms at Lausanne (91%), at Bicêtre (84%, personal data), in Austria (85%) (9), and in Amsterdam (63%) (1). Moreover, two/three of the CTX-M

TABLE 3. MICs of β-lactams for ESBL-positive isolates

β-Lactam(s)	MIC range (μg/ml) for isolates producing ESBL:						
	CTX-M-1	CTX-M-15	CTX-M-2	CTX-M-9	CTX-M-14	TEM-ES	SHV-ES
Amoxicillin	>256	>256	>256	>256	>256	>256	>256
Amoxicillin and clavulanic acid[a]	16	8–32	8	8–>256	4–8	2–8	4–256
Ticarcillin	>256	>256	>256	>256	>256	>256	>256
Ticarcillin and clavulanic acid	128	32–128	64	32	32–64	16–256	16–256
Piperacillin	>256	128–256	128	64–128	32–256	32–>256	32–>256
Piperacillin and tazobactam[b]	8	2–32	2	2–16	1–16	1–16	1–64
Cephalothin	>256	>256	>256	>256	>256	256	>256
Cefoxitin	8	4–32	4	8–32	4–16	4–16	4–256
Ceftazidime	4–8	1–>256	1	1	0.5–2	4	0.25–128
Cefotaxime	>256	128–256	128	32–128	32–64	0.12–128	2–128
Cefepime	32–128	8–128	16	2–8	2–8	0.25–32	0.25–8
Aztreonam	64–256	2–256	16	2–4	2–8	2–16	2–128
Imipenem	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Ertapenem	0.12	0.003–0.5	0.06	0.06–1	0.003–0.12	0.06	0.06–0.5

[a] Clavulanic acid was at a fixed concentration of 4 μg/ml.
[b] Tazobactam was at a fixed concentration of 4 μg/ml.

producers were *E. coli*. The prevalence of CTX-Ms among ESBL-positive *K. pneumoniae* strains was also similarly high in Lausanne (76%) and in Paris (83%), whereas this value was much lower in Austria (30%) and in Italy (12.3%) (20).

Most ESBL-producing *E. coli* isolates (62%) have been isolated from urinary tract infections, as found in other studies (4, 23, 29). More than one-third of CTX-M-producing isolates were from community-acquired infections, as reported from other European countries (3, 4, 22).

Most of the CTX-Ms identified in Switzerland belonged to the CTX-M group 1 (CTX-M-1, ...) as reported in France (Bicêtre Hospital [personal data]) and especially in central and southern France (17), in Austria (9), and in Amsterdam (1). CTX-M-15 was the predominant CTX-M in Lausanne (94%), as elsewhere in Western Europe (4, 17, 20, 29). Twenty percent of the CTX-M enzymes were CTX-M-9-like in Lausanne, 26% were CTX-M-9-like in Austria, and only 11% were CTX-M-9-like in Bicêtre Hospital. Moreover, CTX-M-9 and the genetically related CTX-M-14 were the most prevalent CTX-M types isolated from clinical samples in Spain until 2004 (3).

PFGE analysis showed that the spread of bla_{CTX-M}-positive *E. coli* and *K. pneumoniae* isolates was not related to the spread of single clones as reported in several studies from other countries (United Kingdom, France, Italy, and Spain) (8, 20, 21, 29).

Concerning the non-β-lactam antimicrobial susceptibilities of CTX-M producers, high rates of resistance were observed especially for ciprofloxacin (50%), as reported in other countries such as Austria (49% [9]), Canada (66% [22]), and Italy (73% [20]). However, the rate of resistance to ciprofloxacin of isolates from pediatric patients was lower (11%) than that of isolates from adults (59%), which is consistent with a low usage of fluoroquinolones in pediatric cases.

We identified here the spread of community-acquired CTX-M producers in Switzerland, with CTX-M-15 being the most prevalent type as observed now in other European countries. One of the most interesting findings of this study may be the size of the reservoir of CTX-M producers in pediatric patients. Finally, the spread of CTX-M producers in community-acquired *E. coli* infections in Switzerland in a manner similar to that observed in neighboring countries may indicate difficulty in controlling these emerging resistance determinants whatever the antibiotic policy is.

REFERENCES

1. **Al Naiemi, N., A. Bart, M. D. de Jong, C. M. Vandenbroucke-Grauls, P. J. Rietra, Y. J. Debets-Ossenkopp, P. C. Wever, L. Spanjaard, A. J. Bos, and B. Duim.** 2006. Widely distributed and predominant CTX-M extended-spectrum β-lactamases in Amsterdam, The Netherlands. J. Clin. Microbiol. **44:** 3012–3014.
2. **Bonnet, R.** 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. **48:**1–14.
3. **Bou, G., M. Cartelle, M. Tomas, D. Canle, F. Molina, R. Moure, J. M. Eiros, and A. Guerrero.** 2002. Identification and broad dissemination of the CTX-M-14 β-lactamase in different *Escherichia coli* strains in the northwest area of Spain. J. Clin. Microbiol. **40:**4030–4036.
4. **Brigante, G., F. Luzzaro, M. Perilli, G. Lombardi, A. Coli, G. M. Rossolini, G. Amicosante, and A. Toniolo.** 2005. Evolution of CTX-M-type β-lactamases in isolates of *Escherichia coli* infecting hospital and community patients. Int. J. Antimicrob. Agents **25:**157–162.
5. **Bruderer, T., M. Jutzi, H. Adler, and R. Frei.** 2006. The CTX-M is the most predominant group of extended-spectrum β-lactamases (ESBL) in Switzerland, p. 130. Swiss Soc. Microbiol. 65th Annu. Assembly. Lausanne, Switzerland, 7 and 8 March 2006.
6. **Canton, R., and T. M. Coque.** 2006. The CTX-M β-lactamase pandemic. Curr. Opin. Microbiol. **9:**466–475.
7. **Clinical and Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing; 15th informational supplement. M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Eckert, C., V. Gautier, M. Saladin-Allard, N. Hidri, C. Verdet, Z. Ould-Hocine, G. Barnaud, F. Delisle, A. Rossier, T. Lambert, A. Philippon, and G. Arlet.** 2004. Dissemination of CTX-M-type β-lactamases among clinical isolates of *Enterobacteriaceae* in Paris, France. Antimicrob. Agents Chemother. **48:**1249–1255.
9. **Eisner, A., E. J. Fagan, G. Feierl, H. H. Kessler, E. Marth, D. M. Livermore, and N. Woodford.** 2006. Emergence of *Enterobacteriaceae* isolates producing CTX-M extended-spectrum β-lactamase in Austria. Antimicrob. Agents Chemother. **50:**785–787.
10. **Filippini, M., G. Masiero, and K. Moschetti.** 2006. Socioeconomic determinants of regional differences in outpatient antibiotic consumption: evidence from Switzerland. Health Policy **78:**77–92.
11. **Jarlier, V., M. H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
12. **Karim, A., L. Poirel, S. Nagarajan, and P. Nordmann.** 2001. Plasmid-mediated extended-spectrum β-lactamase (CTX-M-3 like) from India and gene association with insertion sequence IS*Ecp1*. FEMS Microbiol. Lett. **201:**237–241.
13. **Kieser, T.** 1984. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid **12:**19–36.
14. **Knothe, H., P. Shah, V. Krcmery, M. Antal, and S. Mitsuhashi.** 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection **11:**315–317.
15. **Lartigue, M. F., N. Fortineau, and P. Nordmann.** 2005. Spread of novel expanded-spectrum β-lactamases in *Enterobacteriaceae* in a university hospital in the Paris area, France. Clin. Microbiol. Infect. **11:**588–591.
16. **Lartigue, M. F., L. Poirel, and P. Nordmann.** 2004. Diversity of genetic environment of *bla*~CTX-M~ genes. FEMS Microbiol. Lett. **34:**201–207.
17. **Lavigne, J. P., H. Marchandin, J. Delmas, N. Bouziges, E. Lecaillon, L. Cavalie, H. Jean-Pierre, R. Bonnet, and A. Sotto.** 2006. *qnrA* in CTX-M-producing *Escherichia coli* from France. Antimicrob. Agents Chemother. **50:**4224–4228.
18. **Medeiros, A. A.** 1997. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clin. Infect. Dis. **24**(Suppl.):S19–S45.
19. **Moland, E. S., N. D. Hanson, J. A. Black, A. Hossain, W. Song, and K. S. Thomson.** 2006. Prevalence of newer β-lactamases in gram-negative clinical isolates collected in the United States from 2001 to 2002. J. Clin. Microbiol. **44:**3318–3324.
20. **Mugnaioli, C., F. Luzzaro, F. De Luca, G. Brigante, M. Perilli, G. Amicosante, S. Stefani, A. Toniolo, and G. M. Rossolini.** 2006. CTX-M-type extended-spectrum β-lactamases in Italy: molecular epidemiology of an emerging countrywide problem. Antimicrob. Agents Chemother. **50:**2700–2706.
21. **Oteo, J., C. Navarro, E. Cercenado, A. Delgado-Iribarren, I. Wilhelmi, B. Orden, C. Garcia, S. Miguelanez, M. Perez-Vazquez, S. Garcia-Cobos, B. Aracil, V. Bautista, and J. Campos.** 2006. Spread of *Escherichia coli* strains with high-level cefotaxime and ceftazidime resistance between the community, long-term facilities, and hospital institutions. J. Clin. Microbiol. **44:** 2359–2366.
22. **Pitout, J. D., P. Nordmann, K. B. Laupland, and L. Poirel.** 2005. Emergence of *Enterobacteriaceae* producing extended-spectrum β-lactamases (ESBLs) in the community. J. Antimicrob. Chemother. **56:**52–59.
23. **Poirel, L., M. Gniadkowski, and P. Nordmann.** 2002. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum β-lactamase CTX-M-15 and of its structurally related β-lactamase CTX-M-3. J. Antimicrob. Chemother. **50:**1031–1034.
24. **Poirel, L., O. Menuteau, N. Agoli, C. Cattoen, and P. Nordmann.** 2003. Outbreak of extended spectrum β-lactamase VEB-1-producing isolates of *Acinetobacter baumannii* in a French hospital. J. Clin. Microbiol. **41:**3542–3547.
25. **Poirel, L., T. Naas, I. Le Thomas, A. Karim, E. Bingen, and P. Nordmann.** 2001. CTX-M-type extended-spectrum β-lactamase that hydrolyzes ceftazidime through a single amino acid substitution in the omega loop. Antimicrob. Agents Chemother. **45:**3355–3361.

26. **Saladin, M., V. T. Cao, T. Lambert, J. L. Donay, J. L. Herrmann, Z. Ould-Hocine, C. Verdet, F. Delisle, A. Philippon, and G. Arlet.** 2002. Diversity of CTX-M β-lactamases and their promoter regions from *Enterobacteriaceae* isolated in three Parisian hospitals. FEMS Microbiol. Lett. **209:**161–168.

27. **Sambrook, J. E., F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

28. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray,**

D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.

29. **Woodford, N., M. E. Ward, M. E. Kaufmann, J. Turton, E. J. Fagan, D. James, A. P. Johnson, R. Pike, M. Warner, T. Cheasty, A. Pearson, S. Harry, J. B. Leach, A. Loughrey, J. A. Lowes, R. E. Warren, and D. M. Livermore.** 2004. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β-lactamases in the UK. J. Antimicrob. Chemother. **54:** 735–743.