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MINIREVIEW

Growing Group of Extended-Spectrum β-Lactamases: the CTX-M Enzymes

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The production of β-lactamases is the predominant cause of resistance to β-lactam antibiotics in gram-negative bacteria. These enzymes cleave the amide bond in the β-lactam ring, rendering β-lactam antibiotics harmless to bacteria. They are classified according to the scheme of Ambler et al. (2) into four classes, designated classes A to D, on the basis of their amino acid sequences, with classes A and C being the most frequently occurring among bacteria. Oxyimino-cephalosporins such as cefotaxime and ceftazidime, which are inherently less susceptible to β-lactamases, were introduced in the early 1980s to treat infections caused by gram-negative bacilli that were resistant to established β-lactams and that produced class A, C, and D β-lactamases. Their repetitive and increased use induced the appearance of resistant strains, which overproduced class C enzymes (42, 72) and/or which produced extended-spectrum β-lactamases (ESBLs), mainly those of class A but also those of class D (19, 61).

Class A ESBLs hydrolyze oxyimino-cephalosporins and aztreonam but not 7-α-substituted β-lactams. They are generally susceptible to β-lactamase inhibitors (clavulanate, sulbactam, tazobactam). According to the functional classification scheme of Bush et al. (23), class A ESBLs are therefore clustered in group 2be, which can be subdivided on the basis of their activities against ceftazidime and cefotaxime as ceftazidimases (higher levels of hydrolytic activity against ceftazidime than against cefotaxime) and cefotaximases (higher levels of hydrolytic activity against cefotaxime than against ceftazidime), respectively (48).

However, class A ESBLs form a heterogeneous molecular cluster comprising β-lactamases sharing 20 to >99% identity. The earliest class A ESBLs, which were reported from 1985 to 1987, differed from widespread plasmid-mediated TEM-1/2 and SHV-1 penicillinases by one to four point mutations, which extend their hydrolytic spectra (51, 90, 91). TEM and SVH ESBLs now comprise at least 130 members and have a worldwide distribution. Most of them are ceftazidimases, and only a few are cefotaximases.

More recently, non-TEM and non-SHV plasmid-mediated class A ESBLs have been reported: ceftazidimases of the PER, VEB, TLA-1, and GES/IBC types and cefotaximases of the SFO-1, BES-1, and CTX-M types (8, 12, 13, 16, 31, 58, 62, 75, 76, 79, 81, 88, 96). The CTX-M β-lactamases are the most widespread enzymes. They were initially reported in the second half of the 1980s, and their rate of dissemination among bacteria and in most parts of the world has increased dramatically since 1995. This review focuses on the origin, epidemiology, clinical impact, enzymatic properties, and structural relationships of the CTX-M-type ESBLs.

CHRONOLOGY OF CTX-M EMERGENCE

In Japan in 1986, Matsumoto et al. (57) discovered a non-TEM, non-SHV ESBL, designated FEC-1, in a cefotaxime-resistant *Escherichia coli* strain isolated from the fecal flora of a laboratory dog which was used for pharmacokinetic studies of β-lactam antibiotics. In Germany at the beginning of 1989, Bauernfeind et al. (10) reported on a clinical cefotaxime-resistant *E. coli* strain which produced a non-TEM, non-SHV ESBL, designated CTX-M-1, in reference to its hydrolytic activity against cefotaxime. At the same time, an explosive dissemination of cefotaxime-resistant *Salmonella* strains began in South America (9, 80). The β-lactamases responsible for the resistance to cefotaxime observed in these different areas had alkaline pI values, conferred a higher level of resistance to cefotaxime than to ceftazidime, and exhibited a great hydrolytic activity against cefotaxime and susceptibility to inhibitors.

In 1992, the same type of ESBL was reported in clinical *E. coli* strain MEN, isolated at the beginning of 1989 in France from a patient who was an Italian national (13). In the same year, Barthélémy et al. (8) sequenced this enzyme, designated MEN-1, which presented only 39% identity with the TEM and SHV enzymes (http://www.lahey.org/Studies/). It was the first sequence of a plasmid-mediated class A non-TEM, non-SHV ESBL. A few years later, Ishii et al. (47) reported on a MEN-1-related enzyme (83% homologous), designated Toho-1, which was produced by a cefotaxime-resistant *E. coli* strain isolated in 1993 in Japan. The sequencing of two non-TEM, non-SHV ESBL-encoding genes in 1996 revealed that CTX-M-1 is identical to MEN-1 and is a variant of Toho-1, designated CTX-M-2 in a cefotaxime-resistant *Salmonella* strain isolated in Argentina in 1990 (11). In Poland, Gniadkowski et al. (41) identified a variant of CTX-M-1, designated CTX-M-3, in different members of the family *Enterobacteriaceae* isolated in 1996. A few years later, sequencing of the FEC-1-encoding gene (GenBank accession number AB098539) showed that the FEC-1 enzyme differs from the CTX-M-3 enzyme by only two substitutions in the signal peptide. Since then, the CTX-M

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enzymes have formed a rapidly growing family of ESBLs distributed both over wide geographic areas and among a wide range of clinical bacteria, in particular, members of the family of *Enterobacteriaceae*. They are also in environmental species of genus *Kluyvera*, from which they have been characterized by investigation of the mechanisms of natural resistance to β-lactams (33, 45, 78).

PHYLOGENIES AND ORIGINS OF CTX-M ENZYMES

At present, the CTX-M family comprises 40 enzymes. The most closely related β-lactamases (identities, 62 to 75%) are the chromosomal class A enzymes of *Serratia fonticola*, *Klebsiella oxytoca*, *Proteus vulgaris*, and *Citrobacter koseri* (3, 8, 68–70, 82).

CTX-M enzymes can be subclassified by amino acid sequence similarities (17). Figure 1 shows a dendrogram constructed by the neighbor-joining method on the basis of the peptide alignment (94). The phylogenic study reveals five major groups of acquired CTX-M enzymes (the members of each group share >94% identity, whereas ≤90% identity is observed between the members belonging to distinct groups). (i) The CTX-M-1 group includes six plasmid-mediated enzymes (CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-12, CTX-M-15, and FEC-1) (8, 41, 49, 50, 57, 63) and the unpublished enzymes CTX-M-22, CTX-M-23, and CTX-M-28 (GenBank accession numbers AY080894, AF488377, and AJ549244, respectively). (ii) The CTX-M-2 group includes eight plasmid-mediated CTX-M enzymes (CTX-M-2, CTX-M-4, CTX-M-4L, CTX-M-5, CTX-M-6, CTX-M-7, CTX-M-20, and Toho-1) (11, 20, 39, 40, 47, 86, 92). (iii) The CTX-M-8 group includes one plasmid-mediated member (17). (iv) The CTX-M-9 group includes nine plasmid-mediated enzymes (CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16, CTX-M-17, CTX-M-19, CTX-M-21, CTX-M-27, and Toho-2) (14, 15, 26, 29, 53, 55, 64, 74, 85, 86) and two unpublished enzymes (CTX-M-24 [GenBank accession number AY143430] and CTX-M corresponding to accession no. JP0074). (v) The CTX-M-25 group includes the CTX-M-25 and CTX-M-26 enzymes (GenBank accession numbers AY157676 and AF518567, respectively).

The natural CTX-M enzymes of *Kluyvera ascorbata*, designated KLUA enzymes, are clustered in the CTX-M-2 group (45). The KLUA-2 enzyme of *K. ascorbata* strain IP15.79 is identical to the CTX-M-5 enzyme characterized in a *Salmonella enterica* serovar Typhimurium strain (20, 45). The natural β-lactamase KLUG-1 of *Kluyvera georgiana* strain CUETM4246-74 (78) clusters with the CTX-M-8 enzyme and differs from the CTX-M-8 enzyme by only one substitution in the mature form plus two other substitutions within the signal peptide. These relationships of amino acid sequences between the natural enzymes of *Kluyvera* strains and acquired CTX-M enzymes suggest that the natural β-lactamases of *K. ascorbata* and *K. georgiana* are the progenitors of the CTX-M-2 and CTX-M-8 groups, respectively. A natural CTX-M enzyme has also been characterized from *Kluyvera cryocrescens* (33). This β-lactamase, designated KLUC-1, shares only 85 to 86% identity with the most closely related enzymes, which belong to the β-lactamases of the CTX-M-1 group. The KLUC-1 enzyme may therefore form a sixth CTX-M group, the enzymes of which have not yet emerged in clinical strains. The progenitors of the CTX-M-1, CTX-M-9, and CTX-M-25 groups may therefore be unknown at present.

GENETIC PROPERTIES

Genetic support and environment of natural $bla_{\text{CTX-M}}$ genes. The $bla_{\text{CTX-M}}$ genes have been described or presumed to be natural and chromosome mediated in the species *K. cryocrescens* ($bla_{\text{KLUC-1}}$), *K. ascorbata* (bla_{KLUA}), and *K. georgiana* ($bla_{\text{KLUG-1}}$). The DNA sequences surrounding these β-lactamase genes exhibited similarities (33, 45, 78). Open reading frames (ORFs), designated Orf1 and encoding a putative aspartate aminotransferase, have been identified upstream of the bla_{KLUA} and $bla_{\text{KLUC-1}}$ genes and share 96% identity (Fig. 2A). In the intergenic region located immediately upstream of the three natural $bla_{\text{CTX-M}}$ genes, 87 to 90% nucleotide identity has been found. No *ampR* gene has been detected, whereas this Lys-type gene regulates some enzymes related to CTX-M enzymes (the natural β-lactamases of *Serratia marcescens*, *P. vulgari*, and *C. koseri*). ORFs, designated Orf3 and encoding a putative protein that shares 69% identity, have been detected downstream of the bla_{KLUA} and $bla_{\text{KLUC-1}}$ genes.

Genetic support of acquired $bla_{\text{CTX-M}}$ genes. In clinical strains, CTX-M-encoding genes have commonly been located on plasmids that vary in size from 7 kb (26) to 160 kb (50, 64). The $bla_{\text{TEM-1}}$ gene often coexists on the same plasmid; and associations with $bla_{\text{TEM-2}}$, $bla_{\text{OXA-1}}$-type, and bla_{SVH}-type genes are probable (49, 85). These plasmids can also carry genes for resistance to multiple other antibiotics, including aminoglycosides, chloramphenicol, sulfonamide, trimethoprim, and tetracycline.

CTX-M-encoding plasmids are often transmissible by conjugation in vitro. Their frequency of transfer varies from 10^{-7} to 10^{-2} per donor cell. This property explains the easy dissemination of $bla_{\text{CTX-M}}$-harboring plasmids. For example, the same *Pst*I restriction type of a $bla_{\text{CTX-M-3}}$-harboring plasmid has been observed in seven different species of the family *Enterobacteriaceae* in Poland (5, 65). In contrast, 7-kb $bla_{\text{CTX-M-17}}$-harboring plasmid pI843, which has been completely sequenced, does not possess the genes required for conjugation (26). However, the non-self-transmissible plasmids can usually transfer by transformation or can be mobilized (92).

Identical $bla_{\text{CTX-M}}$ genes that are geographically and temporally clustered have been observed on different genetic supports. A $bla_{\text{CTX-M-3}}$ gene has been characterized on plasmids exhibiting two distinct *Pst*I restriction types in Warsaw, Poland (65). The same type of observation has been reported for the same gene in Peking, China (97); for the $bla_{\text{CTX-M-8}}$ gene in Rio Janeiro, Brazil (17); and for the $bla_{\text{CTX-M-14}}$ gene in the Juan Canalejo Hospital in A Coruña, Spain (18). The last gene has been observed in China on a *Bam*HI- and *Eco*RI-restricted fragment located on plasmids ranging in size from 60 to 150 kb, as well as on the chromosomes of *E. coli* and *Enterobacter cloacae* strains (29). Secondary chromosomal insertions of $bla_{\text{CTX-M}}$ genes have also been observed in Japan in clinical *E. coli* strain HK56, which harbored three copies of the gene (100). These data suggest the mobility of $bla_{\text{CTX-M}}$ genes,

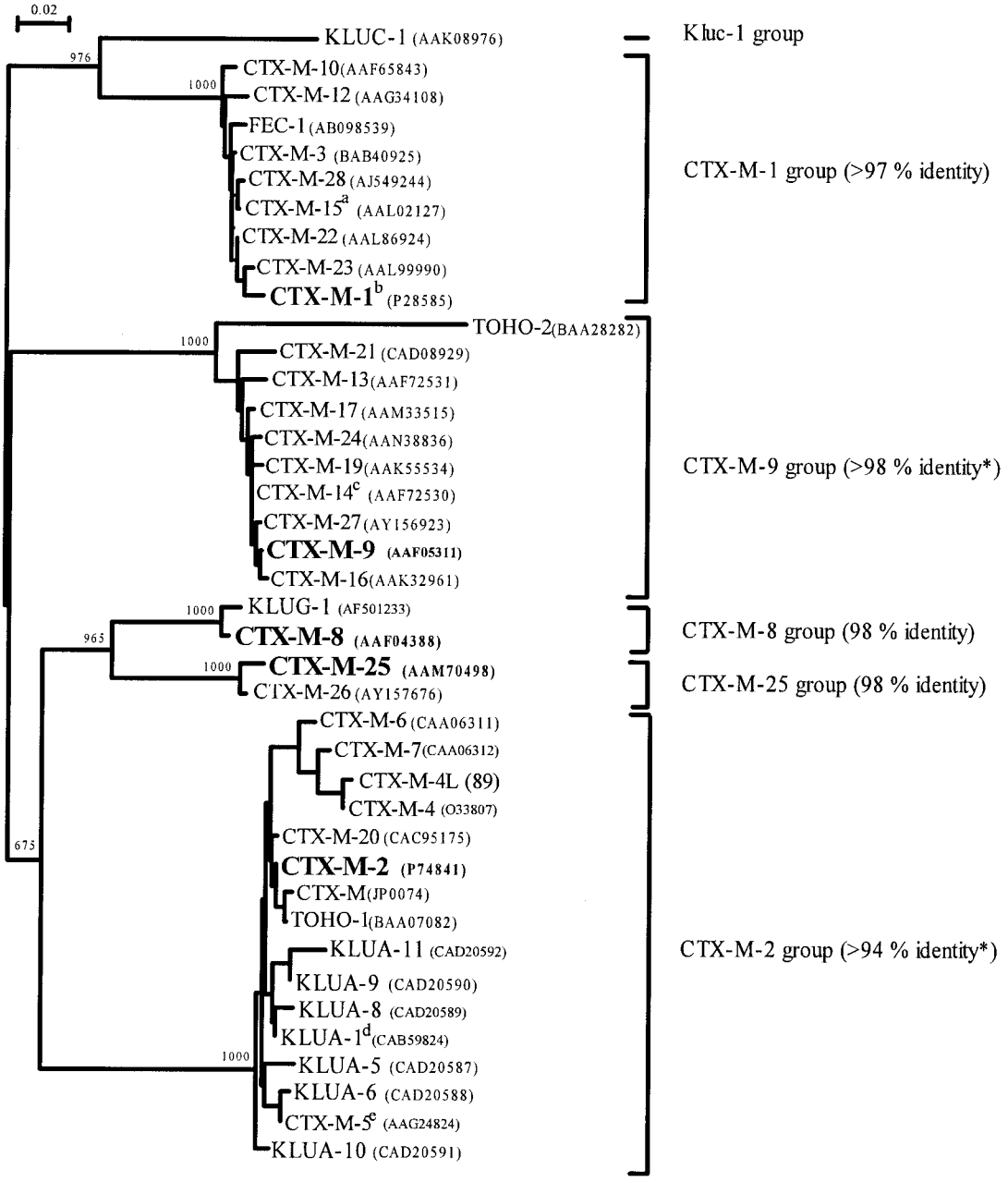

FIG. 1. Dendrogram of CTX-M family. Branch lengths are scaled according to the amino acid changes (94). The numbers at the major branch points refer to the number of times that a particular node was found in 1,000 bootstrap replications. [a], designated UOE-1 and CTX-M-11 in GenBank (accession numbers AY013478 and AJ310929, respectively); [b], designated MEN-1 (8)(GenBank accession number AAB22638); [c], designated CTX-M-18 (74), UOE-2, and Toho-3 (GenBank accession numbers AF325133, AF311345, and AB038771, respectively); [d], encoded by the bla_{KLUA-1}, bla_{KLUA-3}, bla_{KLUA-4}, and $bla_{KLUA-12}$ genes; [e], designated KLUA-2 (45) (GenBank accession number AJ251722); *, the Toho-2 sequence was included i the CTX-M-9 group for the reasons described in references 52 and 54.

which allows their transfer between plasmids and from plasmids to chromosomes.

Genetic environment of acquired bla_{CTX-M} genes. Genetic arguments show the mobilization of natural bla_{CTX-M} genes from the *K. ascorbata* and *K. georgiana* chromosomes to plasmids. The sequences upstream and downstream of the plasmid-mediated $bla_{CTX-M-8}$ gene (138 and 34 bp, respectively) share 95 and 94% identities with those surrounding bla_{KLUG-1} on the chromosome of *K. georgiana* (78). Likewise, the sequences surrounding the genes of plasmid-mediated β-lactamases belonging to CTX-M-2 group share 80 to 100% identities with those surrounding the bla_{KLUA} genes on the chromosome of *K. ascorbata* (45). In plasmids pS21 and pMAR-12, a 1,043-bp sequence downstream of $bla_{CTX-M-2}$ shares 99% identity with the chromosomal DNA of *K. ascorbata* and harbors the Orf3 sequence of the species *K. ascorbata* (4, 34).

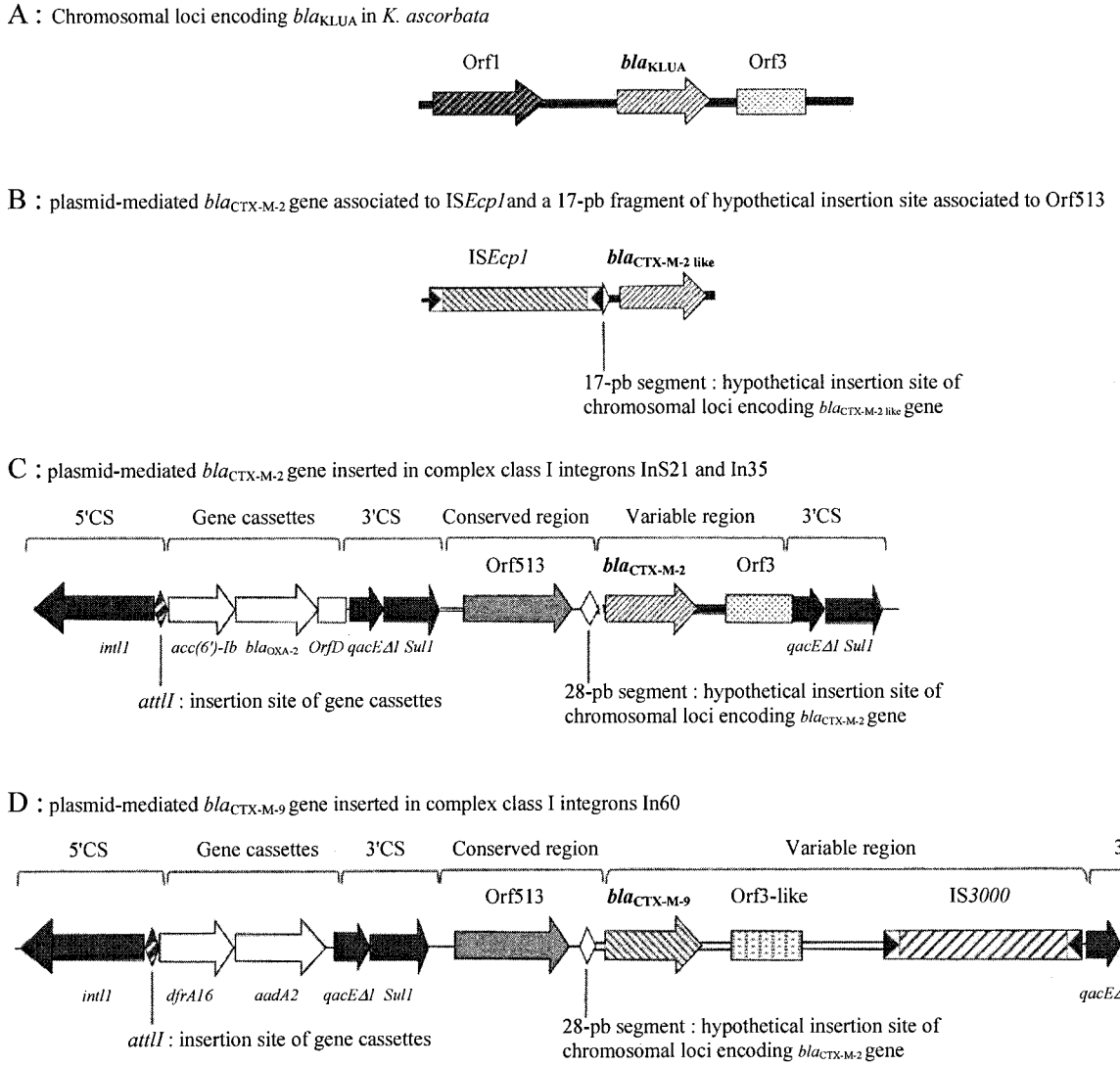

FIG. 2. Comparison of sequences surrounding the bla_{CTX-M} genes in the chromosome of *K. ascorbata* (A) and in plasmid-mediated genes associated with IS*Ecp1* (B) or inserted in complex class I integrons InS21 and In35 (C) and In60 (D) (4, 34, 45, 84, 86). pb, base pairs.

In CTX-M-9-encoding plasmid pMSP071, an 823-bp sequence downstream of the bla_{CTX-M} gene harbors an Orf3-like region that shares 78% identity with Orf3 of *K. ascorbata*, suggesting that $bla_{CTX-M-9}$ and the surrounding sequences could come from a related *Kluyvera* strain (84). Accordingly, it has been suggested that conserved sequences of 42 and 79 bp upstream of the $bla_{CTX-M-1}$ and $bla_{CTX-M-9}$ genes, respectively, are part of the chromosome of a gram-negative rod that could be the progenitor of corresponding clusters (86).

Different elements may be involved in the mobilization of bla_{CTX-M} genes. IS*Ecp1* or IS*Ecp1*-like insertion sequences have repeatedly been observed 42 to 266 bp upstream of ORFs encoding the CTX-M-1, CTX-M-2, CTX-M-3, CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-15, CTX-M-17, CTX-M-19, CTX-M-20, and CTX-M-21 enzymes (1, 18, 29, 36, 49, 86), which is also the case for certain plasmid-mediated *ampC* genes (72, 98) (Fig. 2B). This insertion sequence is composed of two imperfect inverted repeats and an ORF encoding a 420-amino-acid putative transposase. Its amino acid sequence displays only 24% identity with the IS*492* transposase from *Bacteroides fragilis*, the most closely related transposase. Stapleton (P. D. Stapleton, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1457, 1999) suggests that the IS*Ecp1* element is able to achieve the transfer of the downstream DNA sequence by a one-ended transposition process. Plasmid conduction experiments have confirmed the potential involvement of IS*Ecp1* in the mobility of bla_{CTX-M} (26). In addition, the mapping of the $bla_{CTX-M-17}$ promoter region by primer extension has revealed −35 (TTGAAA) and −10 (TA CAAT) promoter sequences at the 3′ end of an IS*Ecp1*-like sequence (nucleotides 2690 to 2719 of GenBank sequence number AY033516) which probably provides the promoter for expression of bla_{CTX-M} genes associated with the IS*Ecp1* element (26, 49).

CTX-M-9- and CTX-M-2-encoding genes have also been observed in unusual class 1 integrons, designated InS21 (plas-

mid pS21) (34), In35 (plasmid pMAR-12) (4), and In60 (plasmid pMSP071) (84) (Fig. 2C and D). These integrons contain the 5′ conserved segment (5′ CS) and a partial or a complete duplication of the 3′ CS. Gene cassettes, which are characterized by 59-BE element-type sequences, are located between the 5′ CS and the first copy of 3′ CS. Between the two 3′ CSs lies a 2,100-bp conserved region that includes Orf513 (previously designated Orf341) and a variable region which harbors no gene cassette. For integrons InS21 and In35, the 2,100-bp conserved region contains a 2,185-bp sequence harboring the $bla_{CTX-M-2}$ gene and Orf3, which probably originated from *K. ascorbata*. For integron In60 (plasmid pMSP071), this variable region contains the $bla_{CTX-M-9}$ gene, an Orf-3-like element, and a putative insertion sequence designated IS*3000*. Other resistance genes have been observed in this variable region, such as the *catA2* gene in the In6 integron, the *dfrA10* gene in the In7 integron, and the *dha-1* gene associated with its *ampR* regulator gene in the integron of plasmid pSAL-1. Hence, it has been suggested that Orf513, the so-called CR (common region) element (67), might encode a putative site-specific DNA recombinase which may have been able to capture genes from the chromosome, like bla_{CTX-M}-harboring loci of *Kluyvera* strains. A conserved 28-bp segment located downstream of Orf513 has been proposed to be a recognition site for the Orf513 putative recombinase. A 17-bp fragment of this segment has been observed downstream of certain IS*Ecp1* sequences (86), suggesting complex mobilization processes involving the Orf513 putative recombinase and the IS*Ecp1* insertion sequence. In addition, Partridge and Hall (67) showed that the CR element (Orf513), the associated variable region, and the second 3′ CS element could be transferred by homologous recombination between 3′ CS elements. Other elements may be involved in the mobilization of bla_{CTX-M} genes, such as IS*10* and IS*26*, partial sequences of which have been observed upstream of $bla_{CTX-M-8}$ (17) and $bla_{CTX-M-1}$ (86), respectively, and an IS*903*-like element observed downstream of the $bla_{CTX-M-14}$ and $bla_{CTX-M-17}$ genes (26, 64).

EPIDEMIOLOGICAL ASPECTS

The chronology of discovery and the distribution of the CTX-M enzymes are provided in Table 1 and are further described below for each geographic region.

South America: Argentina and Brazil. In South America, an explosive dissemination of nontyphoid *Salmonella* strains resistant to cefotaxime was observed beginning in 1989. Starting in a hospital in La Plata, Argentina, the strains spread to neonatology units of pediatric hospitals in Buenos Aires, Argentina, and from there to neighboring countries (10, 71, 80, 81). The $bla_{CTX-M-2}$ gene was characterized from a conjugative plasmid of *S. enterica* serovar Typhimurium strain CAS-5 isolated during this outbreak in 1990 (11). The dissemination of the gene has been suspected or demonstrated in different members of the family *Enterobacteriaceae*, such as *E. coli*, *Shigella sonnei*, *Proteus mirabilis*, *Morganella morganii*, *Citrobacter freundii*, *S. marcescens*, and *Enterobacter aerogenes*; in *Vibrio cholerae*; and in *Aeromonas hydrophila*. The CTX-M-2 enzyme seems to be the most frequent ESBL (75%) in *Enterobacteriaceae* in Argentina (M. Quinteros, M. Mollerach, M. Radice, et al., Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 893, 1999). In Rio de Janeiro, Brazil, of 18 representative ESBL-producing strains of the family *Enterobacteriaceae* collected in 1998 and 1999, CTX-M enzymes ($n = 6$) were the second most frequent ESBLs after SHV β-lactamases ($n = 10$) and were diverse: CTX-M-2 in *P. mirabilis*; CTX-M-9 and CTX-M-16 in *E. coli*; and CTX-M-8 in *Citrobacter amalonaticus*, *E. cloacae*, and *Enterobacter aerogenes* (14, 16, 17).

Far East: Japan, China, Korea, Taiwan, Vietnam, and India. In the Far East, the first clinical strain producing CTX-M enzymes was observed in Japan in 1993 with the characterization of the Toho-1 enzyme from an *E. coli* strain (47). Since then, between 1995 and 2000, five other CTX-M β-lactamases have been isolated from *E. coli* strains in that country: CTX-M-2 (102), CTX-M-3 (102), CTX-M-15 (designated UOE-1 in GenBank [accession number AY013478]), Toho-2 (55), and CTX-M-14 (designated UOE-2 by Muratani et al. [T. Muratani, K. Takahashi, T. Matsumoto, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2004, 2000] and Toho-3 by Ishii et al. [Y. Ishii, M. Galleni, L. Ma, et al., Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1474, 2000]). Surveys of ESBL-producing *Enterobacteriaceae* conducted in Japan showed that the CTX-M-2 and CTX-M-3 enzymes predominate (101, 102). At least three outbreaks involving CTX-M enzymes have occurred in Japan, implicating clonal *E. coli* spread. In one case, the strain was spread by intra- and interhospital dissemination and persisted for 22 months (56, 100; Muratani et al., 40th ICAAC).

In China, CTX-M-3, CTX-M-9, CTX-M-13, and CTX-M-14 enzymes have been reported from *E. coli*, *Klebsiella pneumoniae*, and *E. cloacae* strains. At the Huashan Hospital in Shanghai, China, CTX-M enzymes were the second most frequent ESBLs after SHV enzymes in *K. pneumoniae* (8 of 80) and *E. coli* (13 of 58) strains in 1999 (99) and were the most frequent ESBLs in the *Enterobacteriaceae* in the southern part of China in 1997 and 1998 (29) and in Peking at the Union Medical College Hospital in 1999 (97).

In Taiwan, at the National Cheng Kung University Hospital, a study of ESBL-producing *K. pneumoniae* strains conducted in 1999 revealed the predominance (57.9%) of unrelated CTX-M-3-producing strains (54, 103). Another survey performed in 24 hospitals between 1998 and 2000 showed inter- and intrahospital clonal dissemination of CTX-M-3-producing (28 of 50) and CTX-M-14-producing (22 of 50) *K. pneumoniae* strains (104).

In different parts of Korea, the CTX-M-14 enzyme was also observed in clinical *K. pneumoniae* and *E. coli* strains between 1995 and 1996 and in an *S. sonnei* strain isolated during an outbreak of gastroenteritis in 2000 (54, 64).

In Ho Chi Minh City, Vietnam, CTX-M-14 and a variant designated CTX-M-17 have commonly been observed in *E. coli* and *K. pneumoniae* strains since at least 1996 (26, 27).

In India, a variant of the CTX-M-3 enzyme, designated CTX-M-15, was reported from six unrelated members of the family *Enterobacteriaceae* (four *E. coli* strains, one *K. pneumoniae* strain, and one *E. aerogenes* strain) isolated between April and May 2000 (49).

Eastern Europe: Poland, Latvia, Russia, Greece, Hungary, Bulgaria, Romania, and Turkey. Eastern Europe is another important area affected by the emergence and spread of

TABLE 1. Chronology of discovery and distribution of CTX-M enzymes

Name (pI[a])	Country[b] (yr)	Species	Reference(s)
FEC-1 (8.2)	Japan (1986)	*E. coli*	57
CTX-M-1[c] (8.4)	France/Italy (1989)	*E. coli*, *P. mirabilis*	13, 86
	Germany (1989)	*E. coli*	10
CTX-M-2 (7.9)	Argentina (1992)	Nontyphoid *Salmonella*, *V. cholerae*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Enterobacter* spp., *C. freundii*, *S. marcescens*, *M. morganii*, *E. aerogenes*, *A. hydrophila*, *Shigella flexneri*	9, 71, 80, 80, 81
	Paraguay and Uruguay (1994)	Nontyphoid *Salmonella*	81
	Israel (1992), Paraguay (1994)	*K. pneumoniae*	9
	France (1997)	*P. mirabilis*	86
	Brazil (1998)	*P. mirabilis*	17
	Japan (1998–2000)	*K. pneumoniae*	102
CTX-M-3 (8.4–8.6)	Poland (1996)	*C. freundii*, *E. coli*, *K. pneumoniae*, *K. oxytoca*, *S. marcescens*, *E. cloacae*, *M. morganii*, *S. enterica* serovar Typhimurium	5, 7, 41, 65
	France (1998)	*E. cloacae*	35
	Japan (1998)	*E. cloacae*, *K. pneumoniae*, *S. marcescens*	102
	Taiwan (1998)	*K. pneumoniae*, *E. coli*	103, 104
	Greece (1998)	*E. coli*	59
	China (1999)	*E. coli*, *K. pneumoniae*, *E. cloacae*, *C. freundii*	97
CTX-M-4 (8.4)	Russia (1996), Hungary (1998)	*S. enterica* serovar Typhimurium	38, 40, 92
CTX-M-4L (8.4)	Hungary (1998)	*S. enterica* serovar Typhimurium	93
CTX-M-5[d] (8.8)	Latvia (1996)	*S. enterica* serovar Typhimurium	20
CTX-M-6 (8.4)	Greece (1997)	*S. enterica* serovar Typhimurium	39, 95
CTX-M-7 (8.4)	Greece (1996)	*S. enterica* serovar Typhimurium	39, 95
CTX-M-8 (7.6)	Brazil (1996)	*E. cloacae*, *C. amalonaticus*, *E. aerogenes*	17
CTX-M-9 (7.9–8.1)	France (1994)	*E. coli*	86
	Spain (1996)	*E. coli*, *S. enterica* serovar Virchow	83, 85, 89
	China (1997)	*E. coli*, *K. pneumoniae*, *E. cloacae*	29
	Brazil (1998)	*E. coli*	14
CTX-M-10 (8.1)	Spain (1990)	*K. pneumoniae*, *E. coli*, *E. gergoviae*, *E. cloacae*	24, 30, 63
CTX-M-12 (9.0)	Kenya (1999)	*K. pneumoniae*	50
CTX-M-13 (8.2)	China (1998)	*K. pneumoniae*	29
CTX-M-14[e] (7.9–8.3)	France (1994)	*E. coli*	86
	Korea (1995)	*E. coli*, *K. pneumoniae*, *S. sonnei*	54, 64
	Japan (1996)	*E. coli*	Ishii et al.[f]
	China (1998)	*E. coli*, *K. pneumoniae*, *E. cloacae*	29
	Taiwan (1998)	*E. coli*, *K. pneumoniae*	104
	Brazil (1999)	*E. coli*	Unpublished data
	Spain (2001)	*E. coli*	18
CTX-M-15[g] (8.6)	India (1999)	*E. coli*, *K. pneumoniae*, *E. aerogenes*	49
	Poland (1998)	*E. coli*, *S. marcescens*	6
	Bulgaria (1997–2001)	*E. coli*	I. Schneider et al.[h]
	France (2000), Japan	*E. coli*	Unpublished data
CTX-M-16 (8.2)	Brazil (1996)	*E. coli*	14
CTX-M-17	Vietnam (1996)	*K. pneumoniae*	26, 27
CTX-M-19 (8.0)	France/Vietnam (1999)	*K. pneumoniae*	74
CTX-M-20 (8.3)	France (1998)	*P. mirabilis*	86
CTX-M-21 (8.4)	France (2000)	*E. coli*	86
CTX-M-26	England (2001)	*K. pneumoniae*	21
CTX-M-27 (8.2)	France (2000)	*E. coli*	15
Toho-1 (7.8)	Japan (1993)	*E. coli*	47

Continued on following page

TABLE 1—*Continued*

Name (pI[a])	Country[b] (yr)	Species	Reference(s)
Toho-2 (7.7)	Japan (1995)	*E. coli*	55
KLUC-1 (7.4)	Pasteur Institute (2001P)	*K. cryocrescens* reference strain 79.54	33
KLUA-1[i] (8.0)	Pasteur Institute (2002P)	*K. ascorbata* CIP82:95	45
KLUA-5[j] (8.0)	Pasteur Institute (2002P)	*K. ascorbata* IP12.79	45
KLUA-6 (8.4)	Pasteur Institute (2002P)	*K. ascorbata* IP15–79	45
KLUA-8 (8.0)	Pasteur Institute (2002P)	*K. ascorbata* IP3.89	45
KLUA-9 (8.4)	France (2002P)	*K. ascorbata* TN Ka01	45
KLUA-10 (8.0)	Pasteur Institute (2002P)	*K. ascorbata* IP43.50	45
KLUA-11 (8.4)	Pasteur Institute (2002P)	*K. ascorbata* IP4450.94	45
KLUG-1 (7.6)	DSMZ[k] (2002P)	*K. georgiana* reference strain CUETM4246-74	78

[a] pI, isoelectric point of CTX-M enzyme.
[b] Countries of strain isolation (year of the first isolation or date of publication [P]).
[c] Designated MEN-1 (8) (Genbank accession number AAB22638).
[d] Designated KLUA-2 (45) (Genbank accession number AJ251722).
[e] Designated CTX-M-18 (74), Toho-3, and UOE-2 (Genbank accession numbers AF325133, AF311345, and AB038771, respectively).
[f] Ishii, et al., 40th ICAAC.
[g] Designated UOE-1 and CTX-M-11 in Genbank (accession numbers AY013478 and AJ310929, respectively).
[h] Schneider et al., 12th Congr. Clin. Microb. Infect. Dis.
[i] Encoded by the bla_{KLUA-1}, bla_{KLUA-3}, bla_{KLUA-4}, and $bla_{KLUA-12}$ genes.
[j] designated CTX-M-5 (20) (Genbank accession number AGG24824).
[k] DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

CTX-M enzymes. In Poland, the CTX-M-3 enzyme was characterized from three *C. freundii* strains collected in 1996 (41). In the Praski Hospital in Warsaw, Poland, during a 4-month period between the end of 1996 and the beginning of 1997, the majority (27 of 35) of ESBL-producing strains of the family *Enterobacteriaceae* expressed a CTX-M-3-like enzyme (65). A 4-month survey performed in seven Polish hospitals in 1998 revealed the predominance of an SHV ESBL (60.4%) and similar frequencies of TEM and CTX-M ESBLs (20.8 and 18.8%, respectively). A wider survey undertaken between 1998 and 2000 in 15 hospitals in 10 different cities of Poland revealed the countrywide dissemination of the CTX-M-3 enzyme (5). This great inter- and intrahospital outbreak was due to the clonal spread of a few strains and more particularly to the dissemination of a CTX-M-3-encoding plasmid in *E. coli*, *K. pneumoniae*, *K. oxytoca*, *C. freundii*, *S. marcescens*, *E. cloacae*, and *M. morganii*. *S. enterica* serovar Typhimurium strains harboring a distinct CTX-M-3-encoding plasmid have also been reported recently (7). CTX-M-15, a variant of CTX-M-3 previously described in India, has also been observed in Poland (6) as well as Bulgaria, Romania, and Turkey (I. Schneider, E. Kueleyom, R. Makovska, et al., 12th Congr. Clin. Microbiol. Infect. Dis., abstr. P430, 2002).

In Latvia in 1990, CTX-M-producing *S. enterica* serovar Typhimurium isolates were implicated in a large outbreak of gastroenteritis that involved 4,000 children. The characterization of the CTX-M enzyme from one of these isolates revealed a variant of CTX-M-2, designated CTX-M-5 (20). A small outbreak involving CTX-M-4-producing *S. enterica* serovar Typhimurium strains also occurred in Russia in 1996 (38, 40). The strain involved has been observed in Greece and Hungary (39, 92, 95). These data show the clonal spread of CTX-M-producing *S. enterica* serovar Typhimurium strains in at least three European countries. The enzymes implicated (CTX-M-4, CTX-M-6, and CTX-M-7) were variants of CTX-M-2, like CTX-M-5 observed in the Latvian strain. A possible epidemiological link with the Latvian outbreak has not been investigated. CTX-M-3-producing *E. coli* strains unrelated to those reported in Poland were also isolated in Greece (59).

Western Europe: France, Germany, Spain, and the United Kingdom. The CTX-M enzyme was first characterized in Western Europe in two *E. coli* strains isolated in 1989 in Germany (10) and in France from an Italian patient (13). Since 1989, 11 different CTX-M enzymes have been reported in France from sporadic *E. coli* (CTX-M-1, CTX-M-2, CTX-M-9, CTX-M-14, CTX-M-21, CTX-M-27), *P. mirabilis* (CTX-M-1, CTX-M-2, CTX-M-20), and *E. cloacae* (CTX-M-1, CTX-M-3) isolates (15, 35, 36, 74, 85, 86). However, CTX-M-producing strains were not detected in France until 1998, during a survey of ESBLs, suggesting that the prevalence of CTX-M enzymes was low but probably growing (32).

At the Hospital Ramon y Cajal in Madrid, Spain, the investigation of ESBL-producing *Enterobacter* strains from 1989 to 2000 showed the persistence of a CTX-M-3 variant, designated CTX-M-10, over a 12-year period in unrelated isolates (24, 30, 63). At the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain, the majority (6 of 10) of ESBL-producing *Enterobacteriaceae* isolated between 1994 and 1996 produced CTX-M-9 enzymes (83, 85). In the same area, a CTX-M-9-like enzyme was also observed in three *S. enterica* serovar Virchow strains isolated between 1997 and 1998 (89). In the northwest area of Spain, 50% (17 of 50) of ESBL-producing strains of the family

Enterobacteriaceae isolated in 2001 produced the CTX-M-14 enzyme, the dissemination of which probably occurs by plasmid spread (18).

More recently, the CTX-M enzyme was observed in the United Kingdom from sporadic *K. oxytoca* strains isolated in 2000 and during an outbreak of CTX-M-producing *K. pneumoniae* strains between July 2001 and February 2002 (1, 21).

Although the first CTX-M enzymes were characterized from strains isolated in 1989, their significant expansion started only in 1995. CTX-M enzymes are now endemic over a wide geographic area, including Latin America, the Far East, Europe and the near East (11), North America (60), and Africa (clonal CTX-M-12-producing *K. pneumoniae* strains were isolated in Kenya [50]).

CTX-M-14, CTX-M-3, and CTX-M-2 (Table 1) are the most widespread enzymes. They have been detected in strains isolated from humans and healthy animals, which could be reservoirs of CTX-M-producing strains (22). They are implicated in small, countrywide, or international outbreaks. The emergence and dissemination of these enzymes involve plasmid or strain epidemics, but they also involve mobile elements, like IS*Ecp1*. Most strains producing CTX-M enzymes seem to be implicated in nosocomial infections. However, in contrast to SHV and TEM ESBLs, CTX-M enzymes also emerge in community strains like *V. cholerae*, nontyphoid *Salmonella*, and *Shigella* sp. isolates. This point may have important implications for control of the spread of the CTX-M enzyme. The widespread use of ceftriaxone or cefotaxime has been proposed as a reason for the emergence of CTX-M enzymes (64, 97). Cefepime and cefpirome might also be factors in this process.

The simultaneous observation of CTX-M enzymes in nosocomial and community strains from multiple geographic locations is consistent with their emergence from a widespread reservoir, owing to independent genetic events, like the mobilization of $bla_{\text{CTX-M}}$ genes from the chromosomes of environmental *Kluyvera* bacteria. This process could also explain the delayed expansion of CTX-M enzymes, as opposed to the explosive emergence since 1985 of TEM and SHV ESBLs, which derived from widespread plasmid-mediated enzymes.

PHENOTYPIC CHARACTERISTICS

Phenotype of resistance to β-lactams. Table 2 illustrates the in vitro susceptibilities of *E. coli* transformants and *Kluyvera* species producing CTX-M enzymes. The diversity of MICs could be accounted for in part by experimental variations and more especially by differences in CTX-M-encoding plasmids and *E. coli* hosts. Except in *Kluyvera* species, in which the level of expression of the CTX-M-encoding gene seems very weak, CTX-M β-lactamases usually confer to both wild and laboratory *E. coli* strains high-level resistance to aminopenicillins (ampicillin or amoxicillin), carboxypenicillins (carbenicillin or ticarcillin), ureidopenicillins (piperacillin), and narrow-spectrum cephalosporins (cephalothin, cephaloridine, and cefuroxime). Their susceptibilities to the 7-α-methoxy cephalosporins (cefoxitin) and carbapenems (imipenem and meropenem) were unchanged.

Most enzymes provided a high level of resistance to the oxi-imino cephalosporins cefotaxime and ceftriaxone and variable levels of resistance to cefepime and cefpirome. The MICs of aztreonam were also high. The MICs of ceftazidime increased significantly but often remained in the susceptible range. However, three CTX-M enzymes, CTX-M-15, CTX-M-16, and CTX-M-27, which harbor the Asp240Gly substitution (14, 15, 49, 77), confer eightfold higher levels of resistance to ceftazidime than their parental enzymes, CTX-M-3, CTX-M-9, and CTX-M14, respectively. CTX-M-19, which derived from CTX-M-18 (also designated CTX-M-14) by a Pro167Ser substitution, confers a higher level of resistance to ceftazidime than cefotaxime (74). The recent emergence of these variants suggests that the CTX-M enzymes are evolving toward the acquisition of improved activity against ceftazidime.

The level of resistance to β-lactam–inhibitor combinations depends on the amount of enzyme produced. The MICs of combinations of clavulanate with amoxicillin and ticarcillin vary, and in most cases, susceptibility or a low level of resistance has been observed. Resistance to piperacillin is usually blocked by tazobactam, as is the case for cefotaxime in combination with the inhibitors clavulanate and tazobactam.

Phenotypic detection of CTX-M ESBLs. Ceftazidime resistance is used in practice as an indicator of the presence of ESBLs, and ceftazidime is usually the best substrate for TEM and SHV ESBLs. When used alone this practice could fail to recognize CTX-M-producing strains susceptible to ceftazidime as ESBL producers and therefore greatly hamper the control of the spread of the CTX-M enzyme. Many strains producing ESBLs demonstrate an inoculum effect, in that the MICs of expanded-spectrum cephalosporins rise as the inoculum increases (19). CTX-M-producing isolates, which are not resistant to ceftazidime in vitro, according to European and NCCLS guidelines, could therefore be resistant to ceftazidime in vivo. The use of ceftazidime to treat infections caused by CTX-M-producing strains can therefore lead to therapeutic failure and promote the emergence of CTX-M enzymes with significant hydrolytic activities against ceftazidime. Interpretive antibiograms of CTX-M-producing strains should therefore result in resistance to all expanded-spectrum cephalosporins and aztreonam, whatever the MICs of these molecules are.

In addition to susceptibility to ceftazidime, susceptibility to cefotaxime should also be tested to reduce the risk of overlooking CTX-M production (1, 21). Testing for susceptibility to cefpodoxime, which is hydrolyzed by both TEM and SHV ESBLs and CTX-M ESBLs, is an alternative but might yield a significant number of false-positive results. In both cases, therefore, class A ESBL production should be confirmed by appropriate synergy tests (19).

BIOCHEMICAL PROPERTIES

Mature CTX-M enzymes, except the Toho-2 enzyme, which has 2 fewer residues, contain 291 amino acid residues, which confer a molecular mass of about 28 kDa (53, 55). Their isoelectric points (pIs) range from 7.4 to 9 (Table 1). The kinetic constants (k_{cat} and K_m) of purified CTX-M enzymes are shown in Table 3. CTX-M β-lactamases are less effective against penicillins than TEM and SHV penicillinases, as observed with most class A ESBLs. The highest hydrolytic activity is obtained against narrow-spectrum cephalosporins. The activity against cefotaxime is generally at least 35-fold greater than that against

TABLE 2. β-Lactam MICs for *K. ascorbata*, *K. cryocrescens*, *K. georgiana*, and *E. coli* laboratory strains producing representative CTX-M enzymes

β-Lactam	MIC (μg/ml)													
	Laboratory *E. coli* strains producing:										*Kluyvera* strains producing:			
	CTX-M-3 (77)[a]	CTX-M-15 (77)	CTX-M-4 (40)	CTX-M-5 (20)	CTX-M-9 (14)	CTX-M-16 (14)	CTX-M-14 (15)	CTX-M-27 (15)	CTX-M-18 (74)	CTX-M-19 (74)	CTX-M-8 (17)	KLUA[b] (45)	KLUG-1[c] (78)	KLUC-1[d] (33)
Amoxicillin	>512	>512	>512[e]	>128[e]	>2,048	>512	>512	>512	>512	>512	>512	8	4	64
Amoxicillin + CLA[f]	128	32	8	32	8	8	8	4	128	128	16	0.5	1	<0.5
Ticarcillin	>512	>512	128	>128	>512	>512	>512	>512	>512	>512	>512	64	16	128
Ticarcillin + CLA	64	32		128	16	16	16	8	256	256	32	0.125	1	1
Piperacillin	512	>512	256	>128	256	256	256	256	>512	>512		2	2	<1
Piperacillin + TZP[g]	2	4	2	1	2	2	2	2	16	8		0.125	0.25	<1
Cephalothin	>512	>512			512	512	512	1,024	>512	>512	>512	8	4	8
Cefuroxime	>512	>512	512		512	512	512	512	>512	>512		4	4	16
Cefotaxime	512	512	512	>128	16	16	16	16	64	4	16	0.06	0.25	0.06
Cefotaxime + CLA	2	2			0.06	0.06			0.5	0.5	0.06		0.06	<0.06
Cefpirome	512	512			2	2	2	4			8		0.12	<0.06
Cefepime	128	64					0.5	1	16	4	4	<0.03	0.12	<0.06
Ceftazidime	32	256	2	16	1	8	1	8	2	128	1	<0.03	0.12	<0.06
Ceftazidime + CLA	2	2			0.25	0.25			0.5	16	0.06		<0.06	<0.06
Aztreonam	128	64	32	128	4	8	4	8	64	4	8	0.06	0.06	0.06
Aztreonam + CLA					0.06	0.06			0.25	0.12	0.12			
Moxalactam	0.5	1							0.5	2			<0.06	0.03
Cefoxitin	2	4		16					4	4	4	1	0.06	1
Imipenem	0.25	0.25		0.5					0.25	0.25	0.25	0.125	0.06	0.06

[a] Reference numbers are given in parentheses.
[b] Modal MICs for 12 *K. ascorbata* strains.
[c] MICs for *K. cryocrescens.*
[d] MICs for *K. georgiana.*
[e] MIC of ampicillin.
[f] CLA, clavulanate at a fixed concentration of 2 μg/ml.
[g] TZB, tazobactam at a fixed concentration of 4 μg/ml.

ceftazidime, as observed with the plasmid-mediated SFO-1 enzyme (58, 68). Weak enzymatic efficiencies against ceftazidime distinguish most CTX-M enzymes from TEM and SHV cefotaximases (i.e., TEM-3, TEM-4, SHV-2, and SHV-3). CTX-M enzymes exhibit significant hydrolytic activities against cefepime and cefpirome. No hydrolytic activity against the 7-α-substituted β-lactams (cefoxitine) and imipenem has been detected, as is the case for most class A ESBLs.

Amino acid positions 240 and 167 seem to be involved in the evolution of CTX-M enzymes. CTX-M-15, CTX-M-27, and CTX-M-16, which derive from CTX-M-3, CTX-M-9, and CTX-M-14, respectively, by a Gly240Asp substitution, have greater catalytic efficiencies against ceftazidime (14, 15, 77). The BES-1 cefotaximase, which harbors the same residue (Gly) at position 240, exhibits similar behavior (16). The PER-1 ESBL, which has good catalytic efficiency against ceftazidime, also contains a Gly240 residue (62). CTX-M-19, which derives from CTX-M-18 by a Pro167Ser substitution, is an atypical CTX-M enzyme because of its lower K_m against ceftazidime than against cefotaxime (74). The Pro167Ser substitution in the BPS-1m ESBL, TEM-1, and PSE-4 also improves the activities of the enzymes against ceftazidime (43, 66, 93). However, the Pro167Ser substitution and, to a lesser extent, the Gly240Asp substitution alter the overall hydrolytic activities of CTX-M enzymes.

As observed with most class A ESBLs, CTX-M enzymes exhibit greater susceptibilities to β-lactamase inhibitors. Tazobactam is the most active (50% inhibitory concentrations, 2 to 10 nM for tazobactam and 9 to 90 nM for clavulanate), and sulbactam is the least active (50% inhibitory concentrations, 0.1 to 4.5 μM) (8, 14, 15, 16, 20, 36, 55, 73, 77).

STRUCTURE-FUNCTION RELATIONSHIPS

The overall crystal structure of the Toho-1 enzyme is similar to those of class A non-ESBLs (46). However, the crystal structure of the Toho-1 acyl intermediate in complexes with benzylpenicillin, cephalothin, and cefotaxime has several features which could be involved in the extended-spectrum activities of CTX-M enzymes (87).

Residue Arg276. CTX-M enzymes do not contain the Arg244 residue, which interacts with the carboxylate functions of substrates and inhibitors in most non-ESBLs. This interaction is critical for the catalysis and the inhibition process of the TEM-1 penicillinase (28). The Arg276 residues of CTX-M enzymes were predicted to be a substitute for Arg244. However, the orientation of the tip of Arg276 and its environment are different in CTX-M enzymes and TEM and SHV enzymes (46). Besides, a Arg276Asn mutation in CTX-M-4 did not cause a significant reduction in susceptibility to inhibitors (37), in contrast to the significant reduction in susceptibility observed with an Ser, Cys, Thr, or His substitution of Arg244 in the TEM-1 enzyme (28). On the other hand, relative hydrolysis rates against oxyimino-β-lactams are reduced by substitution of Arg276, lending support to the hypothesis that Arg276 is important for the extension of enzyme activity. However, the Arg276 residue has no interactions with the substrate in acyl intermediate structures (87).

Residues Asn104, Asn132, Ser237, and Asp240. One notable feature concerns residue Ser237 (8), which is thought to be

TABLE 3. Kinetic constants determined with purified CTX-M enzymes[a]

Enzyme	Penicillin G		Amoxicillin		Ticarcillin		Piperacillin		Cephalothin		Cefuroxime		Cefoxitine		Cefotaxime		Cefpirome		Cefepime		Ceftazidime		Aztreonam	
	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)
CTX-M-1	190	11	87	10	15	12			2,450	115	150	75		112[b]	317	125	516	270			1	50[b]		
CTX-M-3	270	2.5	160	185	40	29	180	66	2,800	96	3	49	<0.01		380	113	30	316	0.2	170	<0.01	>3,000	190	188
CTX-M-5	230	50							1,500[c]	350[c]			0.41	0.18	210	95					7.4	440	21	730
CTX-M-8	150	11	55	12	17	14	74	19	1,600	87	35	12		5[b]	72	74	455	1,200	144	990	2	>500	13	800
CTX-M-9	295	25	90	20	60	35	110	20	3,000	150	350	50			450	120	950	800			2	600	10	220
CTX-M-14	290	20	100	20	45	24	200	48	2,700	175	320	40			415	130	940	1,000			3	610	10	200
CTX-M-18	30	29	10	105	3	17	15	23	7[c]	216[c]	40	70	ND[d]	ND	20	54	65	650	20	525	ND	ND	2	286
Toho-2	3.6	6	102	12	14[e]	18[e]	130	84	12,000	470			<0.1	4.8	220	66					1.3	160	0.1	140
CTX-M-15	40	10	20	38	2	5	35	13	35	43	70	13	<0.01		150	54	120	195	10	1,075	2	1,760	1.5	11
CTX-M-16	65	6	40	10	10	13	45	8	2,800	83	500	45			1,400	150	840	520			15	350	3	17
CTX-M-27	11	6	5	10	1	13	9	8	232	83	79	45			113	150	63	520			3	330	0.4	17
CTX-M-19	5	15	1	100	1	30	8	10	30[c]	123[c]	8	40	ND	ND	3	60	ND	ND	ND	ND	0.02	25	ND	ND

[a] Data are from references 8, 14, 15, 16, 20, 36, 55, 73, and 77.
[b] K_m determined as K_i by substrate competition.
[c] Kinetic constant for cephaloridine.
[d] ND, not determinable.
[e] Kinetic constant for carbenicillin.

involved in the extension of the substrate specificities of TEM and SHV ESBLs to cefotaxime (52). The Ser237Ala substitution in the CTX-M-4 enzyme induces a decrease both in relative hydrolytic activity against cefotaxime and in susceptibility to inhibition by clavulanate (40). The acyl intermediate structure of Toho-1 in complex with cefotaxime shows a rotation of the Ser237 side chain, which prevents steric clashes with the methoxyimino group of cefotaxime and which allows the formation of a hydrogen bond with the carboxylate function of cefotaxime (87). Shimamura et al. (87) suggest that this interaction assists in bringing the carbonyl group of the β-lactam ring of cephalosporins to the optimal position in the oxyanion hole for acylation. The relatively low penicillinase activities of CTX-M enzymes may be caused by van der Waals contact between residue Ser237 and the methyl group of the thiazolidine ring.

Asn104, Asn132, Ser237, and Asp240 residues establish hydrogen bonds with the amide and aminothiazole groups of the acyl-amide-cefotaxime chain. This unusual acyl intermediate of CTX-M enzymes in complex with cefotaxime may therefore be involved in the activities of the oxyimino-cephalosporinases by fixing cefotaxime tightly in the binding site (46, 87).

The omega loop. The structure of Toho-1 revealed that the omega loop has fewer hydrogen bond interactions with the β3 strand in the vicinities of Asn170 and Asp240 than the restricted-spectrum β-lactamase of *Bacillus licheniformis*, the enzyme most closely related to Toho-1 at the structural level. No hydrogen bond has been observed between the Phe160 residue and Thr181 and Asp157 residues, which both connect the N and C termini of the omega loop in restricted-spectrum β-lactamases (46). These structural features may increase the flexibility of the omega loop. The structures of acyl intermediates of the Toho-1 enzyme show a shift of the omega loop to helix H5 (87) as a result of a complex structural rearrangement in the hydrophobic core in the vicinity of the omega loop (the residues involved in the rearrangement are Cys69, Ser72, Met135, Phe160, and Thr165). This shift narrows the binding site, but the steric contacts of the Pro167 and Asn170 residues with the aminothiazole ring of cefotaxime are avoided.

Evolution of CTX-M enzymes: residues Ser167 and Gly240. Mutants with point mutations in common CTX-M enzymes exhibiting improved catalytic efficiencies against ceftazidime have recently been observed. The mutations have not previously been observed in natural TEM or SHV ESBLs (52). The CTX-M-15, CTX-M-16, and CTX-M-27 enzymes harbor the Asp240Gly substitution. The presence of Lys and Arg residues at position 240 are known to increase the enzymatic activities of the TEM and SHV ESBLs against ceftazidime (52). The Lys and Arg residues are positively charged and can form an electrostatic bond with the carboxylic acid group on oxyimino substituents of ceftazidime (25, 44). Neutral residue Gly240 is not able to form electrostatic interactions with β-lactams but could favor the accommodation of the oxyimino-ceftazidime side chain (14, 15).

CONCLUSIONS

The CTX-M enzymes, like the TEM and SHV-type ESBLs, emerged in the late 1980s, a few years after the introduction of cefotaxime as a treatment for microbial infectious. Although the worldwide expansion of CTX-M-producing strains was not observed until 1995, it is now a major concern in certain areas, such as South America, the Far East, and Eastern Europe. The progenitors of their genes are the chromosomal *bla* genes of the species *K. ascorbata*, *K. georgiana*, and *K. cryocescens* and of other unknown related strains of the family *Enterobacteriaceae*. Different elements, including IS*Ecp1* and Orf513, may be implicated in the transfer of these genes.

CTX-M enzymes confer higher levels of resistance to cefotaxime than to ceftazidime. The MICs of ceftazidime are sometimes found to be in the susceptible range, whereas ESBL detection is frequently based on ceftazidime utilization. This argues in favor of further studies on how to adapt ESBL detection procedures to survey and control the spread of CTX-M enzymes.

The cefotaximase activity of CTX-M cannot be explained only by localized adjustment at the active site, as in TEM and SHV ESBLs, but, rather, it can also be explained by global arrangements of the omega loop and probably of the β3 strand. However, mutants of CTX-M enzymes harboring improved catalytic efficiencies against ceftazidime have recently been observed, suggesting that the enzymes are evolving as a result of ceftazidime selection pressure. The residues implicated in this evolution have never been observed in naturally occurring TEM or SHV ESBLs, suggesting that the CTX-M enzymes probably have a singular evolutionary potential.

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