



CTX-M β -lactamases

Beta-lactamases are bacterial enzymes that are able to hydrolyze beta-lactam antimicrobial drugs. Pressures from widespread use of antimicrobials have led to selection of bacteria producing extended spectrum β -lactamases (ESBL). ESBLs are characterized by their ability to hydrolyze penicillins, early cephalosporins, oxyimino-thiazolyl cephalosporins (including third and fourth generation cephalosporins) and monobactams but not cephamycins or carbapenems. They are inhibited by suicide-inhibitors such as clavulanic acid, tazobactam and sulbactam. These plasmid encoded periplasmic enzymes are often produced by members of Enterobacteriaceae as well as non-fermenters such as *Acinetobacter spp* and *Pseudomonas spp*. ESBLs have originated due to one-seven point mutations in the beta-lactamase (*bla*) genes, usually of the TEM and SHV types. Most of the ESBLs are placed in Bush's group 2be and Ambler's A class. There are over 186 TEM types and 142 SHV types, although not all of them have ESBL phenotype. Several among them are penicillinases or early cephalosporinases (belonging to group 2b) or inhibitor resistant TEMs (belonging to 2br). Some members of OXA-penicillinases (Bush's group 2de, Ambler's class D) have an ESBL phenotype. There are other lesser prevalent ESBLs such as BEL-, BES-, SFO-, TLA-, PER- and VEB-types. Another most notable of non-TEM, non-SHV ESBLs that have gained widespread importance are CTX-M- β -lactamases.

Properties of CTX-M ESBLs:

Discovery:

Most of the early ESBLs were mainly ceftazidimases, with little or no hydrolytic activity against cefotaxime. ESBLs differed in substrate variability, with some enzymes hydrolyzing one or more cephalosporin type over other types. The first enzyme with cefotaximase property but susceptibility to ceftazidime was reported in 1986 in Japan from an *E. coli* isolate obtained from a dog that was used to study cephalosporin pharmacokinetics. It was designated as FEC-1. On subsequent amino acid sequencing, it was identified similar to CTX-M-3 β -lactamase.

The first clinical isolate with a cefotaximase property was reported from an *E. coli* grown from ear discharge of a 4-month child in Munich, Germany in 1989. It was named CTX-M-1 (CTX for cefotaximase and M for Munich). In the same year, another cefotaximase designated as MEN-1 (based on patient's name) was observed in an *E. coli* isolate obtained in France from an Italian patient. It was later found to be identical to CTX-M-1. In 1989, cefotaximases were observed in *S. typhimurium* obtained from patients with meningitis, septicemia and enteritis in Argentina. This enzyme had a different pI value than that of CTX-M-1. Amino acid sequencing showed that it had only 84% homology with that of CTX-M-1 and was designated as CTX-M-2.

In 1993, a cefotaximase was detected in an *E. coli* obtained from urine of a 1-year old child with cystitis in Japan. It was designated as Toho-1 (based on Toho University School of Medicine). In 1995, another *E. coli* isolate with cefotaximase property was found in urine of a patient with colon cancer in Japan. It was designated as Toho-2. Few years later, the amino acid sequencing led to their renaming as CTX-M-44 and CTX-M-45, respectively.

In 1996 CTX-M-3 was identified among *C. freundii* and *E. coli* isolates obtained from urine specimens in Warsaw, Poland. It was subsequently reported from France (1998) and Taiwan (1999). It differs from CTXM-1 in four amino acid positions. CTX-M-4 was isolated in Russia in 1996 from

S. typhimurium isolates from stool specimens of patients with gastroenteritis as well as sewage specimen. CTX-M-9 were reported from *E. coli* obtained from urine specimens from Spain and Brazil in 1996, followed by detection in China (1997). A retrospective study in France suggested that CTX-M-9 producers existed in isolates collected during 1994.

CTX-M-10, which differs from CTX-M-3 by two amino acid substitutions, was discovered in Spain in 1997. CTX-M-14 was detected in a stool isolate of *Shigella sonnei* and in blood isolates of *E. coli* and *K. pneumoniae* in Korea in 1995. This was followed by its detection in China (1997) and France, Taiwan and Brazil (in 1999). It differs from CTX-M-9 by only one amino acid change, at position 231. CTX-M-15 was first described in fecal *E. coli* isolate from India in 1999. It differs from CTX-M-3 in only one amino acid at position 240 (Asp→Gly), which confers increased catalytic activity against ceftazidime. A retrospective study in Poland on Enterobacteriaceae isolates collected during 1998 revealed the presence of CTX-M-15 in *S. marcescens* and *E. coli* strains. It is currently the most widely disseminated CTX-M type.

Organisms producing CTX-M-β-lactamases:

CTX-M-β-lactamases are often observed in clinical isolates of *E. coli* and *K. pneumoniae*. But, they have also been recovered from several members of Enterobacteriaceae such as *Citrobacter spp*, *Enterobacter spp*, *S. marcescens*, *M. morganii*, *Proteus spp*, *Salmonella spp*, *Shigella spp* etc.

They have also been observed among non-lactose fermenting isolates such as *Pseudomonas spp* and *Acinetobacter spp*. CTX-M-1 was observed in sputum isolates of *P. aeruginosa* and *Stenotrophomonas maltophilia* in Greece from patients with cystic fibrosis in 2004. In the same year CTX-M-43 was identified in *P. aeruginosa* and *Acinetobacter spp* in isolates recovered from Bolivia. In 2005, a CTX-M-2 producing *P. aeruginosa* was detected in a hospitalized patient in Brazil. CTX-M-β-lactamases have also been detected in *Aeromonas spp* and *Vibrio spp*. However, their occurrences in non-fermenters are still very less, which is probably because of incompatibility of plasmids in these bacteria or could also be due to difficulty in detecting ESBLs in these isolates.

Classification:

CTX-M β-lactamases are typical ESBLs that belong to Bush's group 2be and Ambler's class A. There are at least 128 CTX-M types that have been described so far. By using amino-acid sequence relatedness, phylogenetic tree of CTX-M β-lactamases has been constructed. They are divided into five clusters (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25). Naming of the cluster was on the basis of the first member of the group that was described.

Few members are shown below:

CTX-M-1 cluster: CTX-M-1, -3, -10, -12, -15, -22, -23

CTX-M-2 cluster: CTX-M-2, -4, -5, -6, -7, -20, -76, -77

CTX-M-8 cluster: CTX-M-8, -40, -63

CTX-M-9 cluster: CTX-M-9, -14, -15, -16, -17, -18, -19

CTX-M-25 cluster: CTX-M-25, -26, -39, -41, -91

Members within a group have >94% amino acid relatedness and ≤90% relatedness across the groups.

Hydrolytic property:

The *bla*_{CTX-M} gene encodes a 291 amino acid enzyme. A single amino acid change in *bla*_{CTX-M} constitutes a new CTX-M type. Most of the CTX-M β-lactamases are resistant to cefotaxime and ceftriaxone while being susceptible to ceftazidime. The active sites of many of these enzymes are not enlarged to accommodate the drug; instead point substitutions leading to specific interactions may be responsible for the improved activity against ceftazidime and cefotaxime. The C7 beta-amino thiazol-oxymino-amide side chain of ceftazidime has a protective effect against most CTX-M beta-lactamases. However, Asp240Gly CTX-M derivatives demonstrate enhanced hydrolytic activity

against this compound. Acquisition of ceftazidime resistance is frequently associated with mutations at positions 167 and 240.

Some enzymes conferring ceftazidime resistance in each of the clusters are:

CTX-M-1 (CTX-M-15, CTX-M-23, CTX-M-28, CTX-M-29, CTX-M-32, CTX-M-33, CTX-M-42)

CTX-M-2 (CTX-M-35 and CTX-M-43)

CTX-M-9 (CTX-M-16, CTX-M-19, and CTX-M-27)

CTX-M-25 (CTX-M-25 and CTX-M-41)

CTX-M-93 has reduced activity against cefotaxime but increased activity against ceftazidime. It has been observed that as these enzymes evolve a broader substrate range, they sacrifice stability.

Origin and evolution:

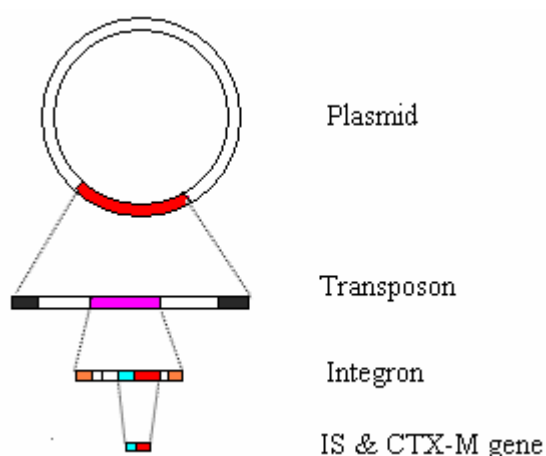
Unlike TEM and SHV enzymes, CTX-M enzymes have not evolved from point mutations in the parent enzymes. They are believed to have originated from the chromosomal *bla* genes of *Kluyvera* *sps* following mobilization into plasmids. Subsequent mutations have contributed to the diversification of the CTX-M family. These mutations also gave the opportunity to expand the hydrolytic activity to ceftazidime. Earlier CTX-M- β -lactamases were cefotaximases, but nowadays more than 60% of CTX-M variants are able to hydrolyze both cefotaxime and ceftazidime.

Kluyvera species are environmental bacteria that are often isolated from water, soil, sewage, and food products of animal origin. They also occur in low counts as commensal in human and animal intestines. They have been found to be responsible for few opportunistic infections and have been isolated from urine and soft tissue infections. CTX-M-1 cluster is believed to have originated from the *bla* gene (*kluC*) of *Kluyvera cryocrescens*. CTX-M-2 cluster is believed to have originated from *kluA* gene of *K. ascorbata*. CTX-M-8, CTX-M-9 and CTX-M-25 clusters are believed to have originated from chromosomal *bla* genes of *K. georgiana* *kluG*, *kluY* and *bla*_{CTX-M-78} respectively. It is believed that CTX-M-74 and CTX-M-75 represents another newer cluster. More recently, it has been suggested that CTX-M-45 also forms a new, separate cluster.

It is believed that recombination events have also helped in evolution and diversification of CTX-M- β -lactamase. It is suspected that CTX-M-64, which was described in a *Shigella sonnei* isolate in Japan from a tourist who had visited China, has evolved out of recombination between CTX-M-14 and CTX-M-15 genes. Such an event is likely to occur in the presence of multiple CTX-M type in the same habitat. CTX-M-64 were isolated from three patients in China, who also harboured isolates with other CTX-M enzymes.

Mobilization and spread:

Even though many *Kluyvera* *spp* contain the *bla*_{klu} genes, they don't express the beta-lactamases and are susceptible to inhibition by cefotaxime as these genes are weakly expressed. The presence of a strong promoter upstream of the *bla* gene results in expression of cefotaxime resistance. Insertion sequences (IS) containing a strong promoter have been observed in clinical isolates exhibiting CTX-M phenotype. These insertion sequences are not found in the natural isolates of *Kluyvera*.



In some cases, the insertion sequences that lie adjacent to *bla*_{CTX-M} gene are found in integrons, which in turn are present in transposons. These transposons may integrate into the plasmids. Various IS identified upstream of *bla*_{CTX-M} genes include *ISEcp1*, *ISCR1*, *IS10* and *IS26*. *ISEcp1*, which was first detected adjacent to a CTX-M-15 gene, is the most widely found IS that is located 42–266 bp upstream of different *bla*_{CTX-M}. It provides a high-level expression promoter for *bla*_{CTX-M}. It has been associated with almost all

clusters except CTX-M-8. ISCR1 has been found in members of CTX-M-2 and CTX-M-9 clusters. IS10 has been found in CTX-M-8 cluster. It is believed that there are nine mobilization events; three in CTX-M-1 cluster, two in CTX-M-2 cluster, two in CTX-M-9 cluster and one each in CTX-M-8 and CTX-M-25 clusters.

Integrative and conjugative elements (ICE) that include ‘conjugative transposons’ and ‘integrated conjugated plasmids’ are diverse group of mobile genetic elements are present in both gram positive and gram negative bacteria. They are usually found integrated into the bacterial chromosome but can excise themselves and reintroduce themselves into another chromosome and transfer between bacterial cells like plasmids. Some of the ICE and IS fail to recognize their own terminal sequences resulting in mobilization of adjacent sequences by a process known as open-end transposition. This is often seen with *ISEcp1* and ISCR1 as well as transposons Tn3 and Tn21.

*bla*_{CTX-M-10} gene has been associated with phage related sequences, which suggests the involvement of bacteriophage in the mobilization of this CTX-M gene.

*bla*_{CTX-M} genes are usually present on large plasmids that also carry additional resistance genes, but have been found on plasmids ranging in size from 7 to 430 kb. The *bla*_{CTX-M} gene is mainly associated with incompatibility group FII plasmids, which are narrow host-range plasmids and are characterized by low copy numbers. These plasmids, which have the propensity to readily acquire and disseminate resistance genes in Enterobacteriaceae members, are termed as ‘epidemic resistance plasmids’. It is believed that IncFII plasmids were widely distributed in Enterobacteriaceae and well adapted to the hosts even before the introduction of antibiotics. Broad host-range plasmids such as IncN, IncI1 and IncL/M have also been involved in the dissemination of CTX-M genes. These plasmids are also known to harbour additional genes conferring resistances to aminoglycosides and fluoroquinolones. IncL/M plasmids have been involved in the spread of *bla*_{CTX-M-3} in Poland and several other Eastern European countries. IncHI2 seems to have preference for *bla*_{CTX-M-9} whereas IncK has preference for *bla*_{CTX-M-14}. The spread of *bla*_{CTX-M-14} in Southern Europe has been mainly on IncK plasmids whereas its spread in Northern Europe has been on IncI1 and IncFII plasmids.

Just as the chromosomal genes have integrated into plasmids, there are reports of the reverse; where plasmid mediated CTX-M-15 genes have been integrated into chromosomes of *E. coli*, *S. enterica* and *K. pneumoniae*.

Clonal dispersion:

Worldwide dissemination of CTX-M enzymes has been helped by the participation of specific *E. coli* and *K. pneumoniae* clones. Despite high diversity among CTX-M producers, a few clones or sequence types (ST) are often found associated with CTX-M enzymes. It is believed that CTX-M genes have successfully adapted in these clones and have led to dissemination of these enzymes. They have been termed as ‘high risk clones’. *E. coli* belonging to ST 131, phylogenetic B group and serotype O25:H4 has been particularly associated with CTX-M-15. This international clone is also associated with CTX-M-1, CTX-M-3 and CTX-M-9, CTX-M-10 and CTX-M-14. It is often seen in community acquired urinary tract infection, but also has been recovered in hospitalized patients as well as from animals. Other lineages such as ST38, ST393 and ST405, which belong to phylogenetic D group, too have been associated with CTX-M enzymes. ST38 and ST393 have been found to be associated with CTX-M-9 and CTX-M-14 genes whereas ST405 has been associated with CTX-M-15, CTX-M-14, and CTX-M-3 genes. CTX-M-associated *E. coli* clones are highly virulent, easily transmitted among family members of colonized or infected patients, and have been found in animals. Most CTX-M enzymes have more frequently been linked to *E. coli* isolates of groups D and B. CTX-M-15-producing strains seems to be more common with groups B2 and D, whereas CTX-M-14 producing strains are more common with phylogenetic groups A, B1, and D. CTX-M-9 producing *E. coli* strains are more commonly linked to groups D and A.

K. pneumoniae clonal complex (CC11) is widely spread in Asia and is associated with CTX-M-14 and CTX-M-15 enzymes. CC11 along with ST15 and ST147 has been responsible for country-wide dissemination of CTX-M-15 producing *K. pneumoniae* in Hungary. A *K. pneumoniae* clone ST16 bearing CTX-M-15 and SHV-28 was responsible for hospital infection in Denmark.

Epidemiology and spread across the world:

Most reports of ESBLs worldwide indicted either TEM or SHV derived enzymes until the early 2000. The first decade of 2000 has seen an explosive spread and high degree of diversification of CTX-M β -lactamases. These enzymes have been observed in *E. coli* isolated from community acquired UTIs and infections in both ICU and non-ICU sections of hospitals worldwide. This has been termed as “CTX-M pandemic”. At present the most widely distributed CTX-Ms are CTX-M-1, CTXM-3, and CTX-M-15 of the group 1 and CTX-M-9 and CTX-M-14 of group 9.

Some studies have shown that dissemination across the globe have occurred due to international travel and immigration. Travel to the Indian Subcontinent and the Middle East is suspected to be associated with high risk of colonization or infection by ESBL producing isolates. Import of animals or animal-derived food contaminated by CTX-M producing bacteria is another route of dissemination. Few studies have revealed simultaneous origins of certain CTX-M types in geographically different locations at the same time period and plasmid studies suggested that they originated independent of each other.

The most prevalent CTX-M-1 cluster has been reported throughout Europe and includes CTX-M-1, CTX-M-3, CTX-M-32, and CTX-M-15. CTX-M group 9 are mostly present in South-Eastern Europe, including Spain, Portugal and United Kingdom. CTX-M-9 and CTX-M-14 are prevalent in Northern Europe. CTX-M-40 and CTX-M-26 have been described in the United Kingdom. In the Canada and the United States CTX-M clusters 1 and 9 are found in clinical isolates of Enterobacteriaceae. Majority of CTX-Ms detected in Canada are CTX-M-15 followed by a minority of CTX-M-14. CTX-M types -14 and -3 are predominant in China. CTX-M-2 and CTX-M-14 were isolated at high frequencies in Japan. Most of CTX-Ms described in India belong to CTX-M-15; the other types being CTX-M-3 and CTX-M-1. CTX-M-2 is the most prevalent CTX-M type across South America. Other reported CTX-M types include CTX-M-31, CTX-M-8, CTX-M-9, CTX-M-12, CTX-M-14, CTX-M-15, and CTX-M-16. CTX-M-15 is the dominant ESBL type among clinical isolates of *E. coli* and *K. pneumoniae* in Australia.

Multi-drug resistance:

Many of the CTX-M ESBL producing isolates are often resistant to fluoroquinolones and aminoglycosides. The IncFII plasmids that bear *bla*_{CTX-M} genes also bear *aac*(6')-Ib-cr gene, which encodes an aminoglycoside- and fluoroquinolone-modifying enzyme. Genes encoding resistance to macrolides, tetracyclines, sulphonamides, trimethoprim, and chloramphenicol have all been associated with *bla*_{CTX-M} containing plasmids. It is believed that fluoroquinolone or multi-drug resistance gives advantage to CTX-M for maintenance due to co-selection process. Some of the CTX-M-15 producers have been found to be resistant to carbapenem, but no mutations are involved in this resistance. It is often due to co-production of other beta-lactamases or loss of porins. Carbapenem resistance in *K. pneumoniae* is often due to loss of OmpK35 and OmpK36 whereas decreased expression of OmpF and OmpC is associated with carbapenem resistance in *E. coli*. Some CTX-M producers have been found to co-produce VIM-1, NDM-1 or OXA-48 carbapenemases. The resistant bacteria tend to become even more resistant by acquiring multi-drug resistant phenotypes; this has been described as ‘genetic capitalism of resistant bacteria’.

Detection of CTX-M- β -lactamases:

Phenotypic detection of CTX-M ESBL producing Enterobacteriaceae isolates is difficult as there are no specific tests to identify CTX-M types. . CTX-M ESBLs have pI values that fall in the range 7.4-9. Because of overlapping isoelectric points, isoelectric focusing is not used in the identification of these enzymes.

Isolates resistant to cefotaxime but susceptible ceftazidime should indicate possible CTX-M type, but many of the CTX-M types can hydrolyze cefotaxime as well as ceftazidime. PCR amplification and sequencing of *bla*_{CTX-M} genes are used to characterize organisms producing CTX-M-β-lactamases.

A multiplex CTX-M PCR was developed, which allowed differentiation between different *bla*_{CTX-M} subtype groups. This method was reported to give non-specific PCR amplification in K1 producing *K. oxytoca* isolates. Another approach is to use a CTX-M-consensus primer-pair to establish a *bla*_{CTX-M}-genotype in Enterobacteriaceae, including *K. oxytoca*. Consensus primers, which recognize almost all of the known variant genes of *bla*_{CTX-M}s, are used to generate an amplified product of 544 bp. Further identification of the specific groups of *bla*_{CTX-M}s, restriction fragment length polymorphism (RFLP) analysis can be employed. However, this method gives false positive amplification with OXY/K1-genes. Hence, PCR alone is not sufficient to detect these enzymes; the amplicons must be subjected to DNA sequencing.

CTX-M consensus primers:

MA1 (F) SCSATGTGCAGYACCAGTAA

MA2 (R) CCGCRATATGRTTGGTGGTG

or

CTX-M-U1 ATGTGCAGYACCAGTAARGTKATGGC

CTX-M-U2 TGGGTRAARTARGTSACCAGAAAYCAGCGG

(R stands for purine, Y stands for pyrimidine, and S stands for G or C)

CTX-1F (F) CCCATGGTTAAAAAATCACTG 891bp

CTX-1R (R) CCGTTTCCGCTATTACAAAC

CTX-2F (F) ATGATGACTCAGAGCATTCGC 893bp

CTX-2R (R) TCGCTCCATTTATTGCATCA

CTX-8F (F) ATGTTAATGACGACAGCCTGTG 689bp

CTX-8R (R) CCGGTTTTATCCCCGACA

CTX-9F (F) GATTGACCGTATTGGGAGTTT 909bp

CTX-9R (R) TATTGAGAGTTACAGCCCTTCG

CTX-25 (F) ATGATGACTCAGAGCATTCG

CTX-25 (R) TGGGTTACGATTTTAGCCGC

Ligation-mediated amplification combined with detection of amplified products on a microarray has been used to detect the various CTX-M groups with 95% specificity and sensitivity.

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Further reading/reference:

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