African Journal of Biotechnology Vol. 6 (7), pp. 831-839, 2 April 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Review

Geographical evolution of the CTX-M β-lactamase – an update

Govinden, U.[1*], Mocktar, C.[1], Moodley, P.[2], Sturm, A. W.[2] and Essack S.Y.[1]

[1]School of Pharmacy and Pharmacology, University of Kwazulu Natal, Durban, 4000.
[2]Nelson R Mandela, School of Medicine, Department of Medical Microbiology, University of Kwazulu Natal, Durban, 4000.

Accepted 23 January, 2007

The CTX-M- type extended spectrum β-lactamases (ESBLs) that preferentially hydrolyze cefotaxime are emerging globally and comprise of more than 50 enzymes. The emergence of novel CTX-M β-lactamases in several countries is noted as opposed to the transfer of established CTX-M genes from one country to another, suggestive of a *de novo* dissemination of CTX-M genes.

Key words: CTX-M β-lactamase, geographic evolution, epidemiology.

INTRODUCTION

Extended spectrum β-lactamases (ESBLs) are molecular class A or D β-lactamases , which are able to hydrolyze oxymino cephalosporins at a rate equal to or higher than 10% of that for benzylpenicillin, have an active-site serine, and are generally inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam or tazobactam. ESBLs are mostly encoded by large plasmids (up to 100 kb and even more) that are transferable from strain to strain and between bacterial species (Stürenburg and Mack, 2003). Hence, ESBLs are an increasingly important cause of resistance to multiple β-lactam drugs in gram-negative bacteria (Livermore and Hawkey, 2005) 'Classical' ESBLs such as TEM (Temoniera) and SHV (sulfhydryl variable) have evolved from the widespread plasmid encoded enzyme families (Stürenburg and Mack, 2003).

Within a few years after its first isolation from an *Escherichia coli* isolate from a Greek patient named Temoniera, the TEM-1 β-lactamase was found worldwide and production of this enzyme is now the most commonly encountered mechanism of resistance to the β-lactam group of drugs in gram-negative bacilli. The first TEM variant with increased activity against extended-spectrum cephalosporins was TEM-3, which was reported in 1987. Since then there has been a rapid increase in the number and variety of extended-spectrum TEM variants (Stürenburg and Mack, 2003). More than 150 TEM derivatives have currently been documented (http:// www.lahey.org/Studies; last assessed 1 December 2006).

The SHV family of β-lactamases appears to have been derived from *Klebsiella* spp. SHV-1, is universally found in *Klebsiella pneumoniae*. In 1983, three strains of *K. pneumoniae* and one strain of *Serratia marcescens* isolated in West Germany were able to transfer resistance to cefotaxime as well as to the newer cephalosporins. This new plasmidic β-lactamase, called SHV-2, was derived from a point mutation in SHV-1. This mutation, at position 238 from glycine to serine resulted in an enhanced affinity of the SHV-1 β-lactamase for oxyimino cephalosporins, with a significant rise in the MIC of cefotaxime and a more limited rise in the MIC of ceftazidime. Subsequently, a number of ESBL variants containing amino acid changes have been reported (Stürenburg and Mack, 2003). There are now over 90 SHV-type enzymes (http://www.lahey.org/Studies; last accessed 1 December 2006).

In 1989, non-TEM, non-SHV, ESBL-producing bacteria expressing a higher level of resistance to cefotaxime than to ceftazidime was described in *E. coli* isolates from Germany. Owing to the high activity against cefotaxime, these new members of the ESBL family were named CTX-M β-lactamases (Rasmussen and Hoiby, 2004). The CTX-M family comprises more than 50 enzymes from various countries as described in Table 1 and can be

*Corresponding author. E-mail: Govindenu@ukzn.ac.za. Tel: + 2731-2608251; Fax + 2731-2607792.

subclassified by amino acid sequence similarities. A phylogenic study reveals five major groups of CTX-M enzymes with the members of each group sharing >94% identity, whereas ≤ 90 % identity is observed between the members belonging to distinct groups (Bonnet, 2004). The five major groups are clusters of CTX-M-1,-2,-8, -9 and -25 (http://www.lahey.org/Studies/, last accessed 1 December 2006).

As a group, the CTX-M-type ß-lactamases are closest in amino acid identity to the chromosomal cephalosporinases of *Kluyvera georgiana*, *Kluyvera cryorescens*, and *Kluyvera ascorbata* (Paterson et al., 2003). The natural CTX-M β-lactamases of *K. ascorbata*, designated KLUA β-lactamases, are clustered in the CTX-M-2 group. The KLUA-2 β-lactamase of *K. ascorbata* strain IP15.79 is identical to the CTX-M-5 β-lactamase characterized in a *Salmonella enterica* serovar Typhimurium strain. The natural ß-lactamase KLUG-1 of *K. georgiana* strain CUETM4246-74 clusters with the CTX-M-8 β-lactamase. These relationships of amino acid sequences between the natural β-lactamase of *Kluyvera* strains and the CTX-M β-lactamase suggest that the natural ß-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 β-lactamase has also been characterized from *K. cryocrescens*. This ß-lactamase, designated KLUC-1, shares only 85 to 86% identity with the most closely related β-lactamases, which belong to the ß-lactamases of the CTX-M-1 group, although an enzyme identitical to CTX-M-3 was isolated from a strain of *K. ascorbata*. The CTX-M-9 group is related to enzymes from *Kluyvera spp*. isolated from Guyana, which were identical to CTX-M-14 (Bonnet, 2004; Pitout et al., 2005a).

Different genetic elements are associated with *bla* CTX-M genes. IS*ECP1*-like insertion sequences are most frequently reported. This insertion sequence element has been found to be associated with four out of the five *bla* CTX-M clusters (CTX-M-1,-2,-9 and -25 clusters) (Lartigue et al., 2006). Many CTX-M genes are located near or within transposons, or within mobile gene cassettes, which could permit rapid dissemination, thus CTX-M-producing strains have a growing distribution and prevalence. South America, Mediterranean and Eastern European countries as well as East Asia account for most reported isolates (Hopkins et al., 2006).

EPIDEMIOLOGY OF CTX-M B-LACTAMASES

America

In Argentina, a nationwide replacement of cefotaxime with ceftriaxone in 1990 co-incided with severe infections, diagnosed with meningitis, septicaemia, and enteritis. The infections emerged in August 1990 and were caused by multiresistant strains of *Salmonella typhimurium* which were resistant to cefotaxime but susceptible to ceftazidime, owing to the production of CTX-M-2 (Rasmussen and Hoiby, 2004). ESBLs were assessed by Patterson et al. (2003) in a collection of 455 isolates of *K. pneumoniae* from 12 hospitals in 7 countries between 1996 and 1997. Of the 18 ESBL positive isolates from Argentina, 11 produced the CTX-M-2 type β-lactamase. An ESBL study of 427 enterobacterial strains identified CTX-M-2 in 19 isolates and CTX-M-31 (a variant of CTX-M-2) in 2 isolates (Quinteros et al., 2003). An ESBL study of 18 Enterobacteriacae strains collected in 1996 and 1997 from hospitals in Brazil identified CTX-M-8 in *Citrobacter amalonaticus* and CTX-M-2 in *Proteus mirabilis* (Bonnet et al., 2000). CTX-M-9 and CTX-M-16 (differing from CTX-M-9 by only 1 amino acid substitution) were observed in 2 of 3 *E. coli* strains from hospitals in Brazil in 1996 (Bonnet et al., 2001). During the period 2000 to 2002 CTX-M-14 ß-lactamases were responsible for a community-wide outbreak in the Calgary Health Region of Canada (Pitout et al., 2005b). In 2004 Abdalhamid et al. reported the presence of CTX-M-30 in *Citrobacter freundii* from 4 different patients in Canada. In 2004 in Colombia, 7 *K. pneumoniae* isolates that were collected from three different hospitals were positive for the CTX-M-1 group and CTX-M-12 was identified in 1 isolate (Villegas et al., 2004). In 2004 Liebana et al. reported CTX-M-15 that was characterized in 2002 from a paediatric patient with a *S. enterica* serotype Infantis infection in Honduras.

Asia

In Japan in 1986, Matsumoto et al. discovered a non-TEM, non-SHV ESBL, in a cefotaxime-resistant *E. coli* strain isolated from the faecal flora of a laboratory dog which was used for pharmacokinetic studies of ß-lactam antibiotics (Bonnet, 2004). A few years later, Ishii et al. (1995) reported on a CTX-M-1-related enzyme, designated Toho-1 (CTX-M-44), which was produced by a cefotaxime-resistant *E. coli* strain isolated from the urine of a patient in 1993 in Japan. In 1998, Ma et al. also reported a cefotaxime resistant *E. coli* isolate containing Toho-2 (CTX-M-45). Surveys of ESBL-producing Enterobacteriaceae in Japan showed that the CTX-M-2 and CTX-M-3 enzymes predominate. At least three outbreaks involving CTX-M enzymes have occurred in Japan, implicating clonal *E. coli* spread (Bonnet, 2004). The molecular types of CTX-M- ß-lactamases in Japan were investigated in 1397 gram-negative bacilli collected between 2001 and 2003. 317 isolates were positive for CTX-M-type ß-lactamases. The investigation revealed that gram-negative nosocomial bacilli producing the CTX-M-1, -2 or -9 group of enzymes had already been dispersed in various clinical settings in Japan, although strains that produce TEM-or SHV-derived ESBLs are infrequently found (Shibata et al., 2006).

The first cefotaximase-producing (CTX-M-3) in a non-clinical *S. enterica* serovar Senftenberg in Japan was reported in 2004. In this study 58 clinical and non-clinical

Table 1. CTX-M-type β-lactamases [a].

ß-Lactamase	Organism	Country	Year of Genbank submission	GenBank Nucleotide accession number
CTX-M-1	*E. coli*	Germany	1995	X92506
CTX-M-2	*S. typhimurium*	Argentina	1995	X92507
CTX-M-3	*C. freundii*	Poland	1996	Y10278
CTX-M-4	*S. typhimurium*	Greece	1997	Y14156
CTX-M-5	*S. typhimurium*	Latvia	1997	U95364
CTX-M-6	*S. typhimurium*	Greece	1998	AJ005044
CTX-M-7	*S. typhimurium*	Greece	1998	AJ005045
CTX-M-8	*C. amalonaticus*	Brazil	1999	AF189721
CTX-M-9	*E. coli*	Spain	1999	AF174129
CTX-M-10	*E. coli*	Spain	2000	AF255298
CTX-M-11	*K. pneumoniae*	China	2000	AY005110
CTX-M-12	*K. pneumoniae*	Africa	2000	AF305837
CTX-M-13	*K. pneumoniae*	China	2000	AF252623
CTX-M-14*	*E. coli*	China	2000	AF252622
CTX-M-15	*E. coli*	India	2001	AY044436
CTX-M-16	*E. coli*	Brazil	2001	AY029068
CTX-M-17	*K. pneumoniae*	France	2001	AY033516
CTX-M-18*	*K. pneumoniae*	France	2000	AF325133
CTX-M-19	*K. pneumoniae*	France	2000	AF325134
CTX-M-20	*P. mirabilis*	France	2001	AJ416344
CTX-M-21	*E. coli*	France	2001	AJ416346
CTX-M-22	*K. pneumoniae*	China	2002	AY080894
CTX-M-23	*E. coli*	Germany	2002	AF488377
CTX-M-24	*K. pneumoniae*	China	2002	AY143430
CTX-M-25	*E. coli*	Canada	2002	AF518567
CTX-M-26	*K. pneumoniae*	United Kingdom	2002	AY157676
CTX-M-27	*E. coli*	France	2002	AY156923
CTX-M-28	*E. coli*	France	2003	AJ549244
CTX-M-29	*E. coli*	China	2003	AY267213
CTX-M-30	*C. freundii*	Canada	2003	AY292654
CTX-M-31	*Providencia spp.*	Argentina	2003	AJ567481
CTX-M-32	*E. coli*	Spain	2003	AJ557142
CTX-M-33	*E. coli*	Greece	2003	AY238472
CTX-M-34	*E. coli*	Spain	2003	AY515297
CTX-M-35	*k. oxytoca*	Japan	2004	AB176534
CTX-M-36	*E. coli*	Japan	2004	AB177384
CTX-M-37	*E. cloacae*	Mongolia	2004	AY649755
CTX-M-38	*K. pneumoniae*	China	2004	AY822595
CTX-M-39	*E. coli*	Israel	2005	AY954516
CTX-M-40	*E. coli*	United Kingdom	2004	AY750914
CTX-M-41	*P. mirbilis*	Israel	2005	DQO23162
CTX-M-42	*E. coli*	Russia	2005	DQO61159
CTX-M-43	*A. baumanni*	Bolivia	2005	DQ102702
CTX-M-44 (Toho-1)	*E. coli*	Japan	1994	D37830
CTX-M-45 (Toho-2)	*E. coli*	Japan	1996	D89862

Table 1. Contd.

CTX-M-46	*K. pneumoniae*	China	2004	AY847147
CTX-M-47	*E. coli*	China	2004	AY847143
CTX-M-48	*K. pneumoniae*	China	2004	AY847144
CTX-M-49	*K. pneumoniae*	China	2004	AY847145
CTX-M-50	*K. pneumoniae*	China	2004	AY847146
CTX-M-51	*E. coli*	Spain	2005	DQ211987
CTX-M-52	*K. pneumoniae*	China	2005	DQ223685
CTX-M-53	*S. enterica*	France	2005	DQ268764
CTX-M-54	*K. pneumoniae*	Korea	2005	DQ303459
CTX-M-55	*E. coli*	China	2005	DQ343292
CTX-M-56		Not released		
CTX-M-57	*S. enterica*	United Kingdom	2006	DQ810789
CTX-M-58		Not released		
UOE-1	*E. coli*	Japan	2000	AY013478

*Amino acid sequences of CTX-M-14 and CTX-M-18 are identical.
[a]Data adapted from Lahey (http://www.lahey.org/Studies/ ; last accessed 1 December 2006) and Genbank (http://www.ncbi.nlm.nih.; last accessed 1 December 2006).

isolates of various *Salmonella* serovars were screened for ESBL production. Only 1 strain of *S. enterica* serovar Senftenberg was isolated from river water in Hiroshima in 1999 and displayed an ESBL phenotype (Ahmed et al., 2004).

In China, CTX-M-3,-9,-13 and-14 type enzymes have been reported from *E. coli*, *K. pneumoniae*, and *Enterobacter cloacae* strains. At the Huashan Hospital in 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 (Bonnet, 2004). Molecular characterization of 57 ESBL strains in a study in 2005 in China revealed that the majority of the strains (94.7%) were CTX-M type, with a predominance of CTX-M-14 and -3 types (Pottumarthy et al., 2005). An ESBL study in the Anhui province in China identified 54 CTX-M positive *E. coli* and *K. pneumoniae* isolates. The isolates contained CTX-M-14 type ß-lactamase with one to three point mutations occurring in eight isolates. The enzymes were designated CTX-M-46,-47,-48,-49 and -50 (Li and Li, 2005; http://www.lahey.org/Studies/; last accessed 1 December 2006).

In Taiwan, at the National Cheng Kung University Hospital, a study of ESBL-producing *K. pneumoniae* strains conducted in 1999 revealed predominance (57.9%) of unrelated CTX-M-3-producing strains. Another survey performed in 24 hospitals between 1998 and 2000 showed inter- and intra-hospital clonal dissemination of CTX-M-3-producing (28 of 50) and CTX-M-14-producing (22 of 50) *K. pneumoniae* strains (Bonnet, 2004). In 2003 Paterson et al., reported the presence of a CTX-M-3 type ß-lactamase in a single *K. pneumoniae* isolate from a hospital in Taiwan. During September 2000 to December 2001 88 ESBL Enterobacteriaceae isolates from the Chang Gung children's hospital in Taiwan produced CTX-M-3 in 52 isolates. This was the most prevalent ESBL. CTX-M-3 was also the most common type of ESBL produced by *E. coli* and *K. pneumoniae* (Wu et al., 2003). Wu et al. also identified CTX-M -3 in 22 out of 34 *S. marcescens* clinical isolates from a medical centre in Taiwan. A study reported in 2006 from seven medical centres in Taiwan, described CTX-M-type ß-lactamases as one of the most prevalent ESBLs. CTX-M- 3, -9, -14, -15, -17, -19, and -38 were identified in this study (Yan et al., 2006).

In different parts of Korea, the CTX-M-14 enzyme was also observed in *K. pneumoniae* and *E. coli* strains between 1995 and 1996 and in a *Shigella sonnei* strain isolated during an outbreak of gastroenteritis in 2000 (Bonnet, 2004). CTX-M-3, -9, -14 and -15 were detected in 41 out of 603 isolates of Enterobacteriaceae collected in 2003 from three university hospitals in Korea (Kim, 2005). In 2004 a nosocomial outbreak of paediatric gastroenteritis in Korea was caused by CTX-M-14 type ESBL producing strains of *S. enterica* serovar London. The isolates had pulsed-field gel electrophoresis patterns identical to those of the previously isolated antimicrobial susceptible strains from community-acquired gastroenteritis, suggesting the susceptible clone acquired the resistance (Yong et al., 2005). A novel ceftazidime-hydrolyzing CTX-M mutant, CTX-M-54, produced by a *K. pneumoniae* isolate in Korea was reported by Bae et al. (2006). CTX-M-14 and a variant designated CTX-M-17 have commonly been observed in *E. coli* and *K. pneumoniae* strains since 1996 in Ho Chi Minh City, Vietnam (Bonnet, 2004).

CTX-M-37 (Genbank accession number AY 649755) was first reported in 2004 in Mongolia from *E. cloacae* cli-

nical isolate (http://www.ncbi.nlm.nih. last accessed 1 December 2006). Five Enterobacteriaceae strains producing CTX-M-2, -9, -11 and -15 were reported in 2004 in Singapore (Koh et al**.,** 2004). In India, CTX-M-15 was reported in 2001 from six non-clonally related members of the family Enterobacteriaceae (Karim et al., 2001).

Europe and Middle East

At a Hospital in Warsaw, Poland, during a 4-month period between 1996 and 1997, the majority (27 of 35) of ESBL-producing strains of the family Enterobacteriaceae expressed a CTX-M-3-like enzyme. 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. This great inter- and intra-hospital 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*, *Klebsiella oxytoca*, *C. freundii*, *S. marcescens*, *E. cloacae*, and *Morganella morganii*. *S. enterica* serovar Typhimurium strains harboring a distinct CTX-M-3-encoding plasmid have also been reported. CTX-M-15, a variant of CTX-M-3 previously described in India, has also been observed in Poland as well as in Bulgaria, Romania, and Turkey (Bonnet, 2004). The CTX-M-2 type ß-lactamase in a single *K. pneumoniae* isolate from a hospital in Turkey was reported by Paterson et al. (2003). A large outbreak of *Salmonella* gastroenteritis that involved 4000 children in Latvia in 1990 was still ongoing when reported in 1998. The majority of cases were associated with *S. typhimurium* strains producing CTX-M β-lactamases. CTX-M-5 was found in one of these strains (Tzouvelekis, 2000).

A small outbreak involving CTX-M-4-producing *S. enterica* serovar Typhimurium strains occurred in Russia in 1996. The strain involved has been observed in Greece and Hungary. Clonal spread of CTX-M-producing *S. enterica* serovar Typhimurium strains in at least three European countries was detected. The enzymes implicated (CTX-M-4,-6, and -7) were variants of CTX-M-2, like the CTX-M-5 observed in the Latvian strain. CTX-M-3-producing *E. coli* strains unrelated to those reported in Poland were also isolated in Greece (Bonnet, 2004). An outbreak of *K. pneumoniae* producing CTX-M-3-type β-lactamases occurred in Novosibirsk in the period 1997 to 1998. The outbreak was caused by a proliferation of 2 major clones. Between 1997 and 1998, nosocomial isolates of *E. coli* and *K. pneumoniae* were collected from 28 Russian hospitals with CTX-M-1 β-lactamase being the most prevalent (Rasmussen and Hoiby, 2004).

The CTX-M enzyme was first characterized in Western Europe in two *E. coli* strains isolated in 1989 in Germany and in France from an Italian patient. In 2004 Stürenburg et al. reported the presence of CTX-M-23 from *E. coli* and *K. pneumoniae* strains which were isolated from a 46-year-old man in Germany during treatment of postoperative peritonitis with ceftazidime (Stürenburg et al., 2004). Since 1989, 11 different CTX-M enzymes have been reported in France from sporadic *E. coli* (CTX-M-1,-2,-9,-14, -18, -19, - 21 and -27), *P. mirabilis* (CTX-M-1,-2 and -20), and *E. cloacae* (CTX-M-1 and -3) isolates (Bonnet, 2004). However, in 2006 Eckert et al. (2006) reported 7 CTX-M-type β-lactamases (CTX-M-1, -2, -3, -9, -14, -15 and -24) among 28 strains of Enterobacteriaceae that were collected from five different hospitals in Paris, France. The emergence and spread of three clonally related virulent isolates of CTX-M-15-producing *E. coli* in a French geriatric hospital was reported in 2004 (Leflon-Guibout et al., 2004).

Paterson et al. (2003) identified 1 CTX-M-2 type ß-lactamase from a *K. pneumoniae* isolate in Belgium in 2003. Three hundred and sixty Enterobacteriaceae and non fermenting gram-negative bacilli isolated during one week in 2004 at 5 hospitals in Netherlands were evaluated for the presence of ESBLs. CTX-M-1,-2,-9 and -15 were found among 18 isolates (Naiemi et al., 2006). At a Hospital in Madrid, Spain, the investigation of ESBL-producing *Enterobacter* strains from 1989 to 2000 showed the persistence of CTX-M-10 over a 12-year period in unrelated isolates. At a Hospital in Barcelona, Spain, the majority (6 out of 10) of ESBL-producing Enterobacteriaceae isolated between 1994 and 1996 produced CTX-M-9 enzymes. 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. In the northwest area of Spain, 50% of ESBL-producing strains of the family Enterobacteriaceae isolated in 2001 produced the CTX-M-14 enzyme (Bonnet, 2004). Four *S. enterica* serovar Virchow strains resistant to broad-spectrum cephalosporins were isolated from patients with gastroenteritis in 1997 and 1998 in Murcia and Barcelona, Spain. The isolates expressed a CTX-M-9 type β-lactamase (Simarro et al., 2000). In 2003 Pagani et al. reported the detection of CTX-M-1,-2 and -15 in 12 out of 232 ESBL producers from a Spanish hospital in Northern Italy. The most prevalent CTX-M ESBLs [CTX-M-9 (27.3%) and CTX-M-14 (20.5%)] were found in *E. coli*, in a nationwide study of *E. coli* (n = 170) and *K. pneumoniae* (n = 70) producing ESBLs in Spanish hospitals in 2005, whilst CTX-M-10 was found in only 3 *K. pneumoniae* isolates (Hernandez et al., 2005). In 2005 a large outbreak was caused by CTX-M-1- producing multiresistant *K. pneumoniae* in a Spanish intensive care unit (51 patients) (Mena et al., 2006). The prevalence and types of genes encoding ESBLs in 642 clinical isolates of Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. were assessed in Bolivia in 2004. 106 out of the 150 putative ESBL producing isolates con-

tained CTX-M-2, and 32 isolates contained CTX-M-43 (Celenza et al., 2006). A study in 2005 reported the detection of CTX-M-1-producing clinical isolates of *C. amalonaticus* and *M. morgani* from an area of Northern Italy where CTX-M producers were found to be widespread in *E. coli*. This study showed that the CTX-M-1 β-lactamase was possibly acquired by these unusual hosts *in vivo*, after co-infection with *E. coli* strains carrying conjugative plasmids bearing the *bla* CTX-M-1 gene (Mugnaioloi et al., 2005). The first description of CTX-M-15 producing *K. pneumoniae* in Portugal was reported in 2005 (Conceicao et al., 2005).

The first outbreak caused by *K. pneumoniae* producing CTX-M-26 was recorded in Birmingham England in 2001 (Livermore and Hawkey, 2005). The United Kingdom (UK) has experienced a sudden rise in ESBL rates, largely due to the appearance and spread of *E. coli* producing CTX-M-15 type β-lactamase. The first significant outbreak of CTX-M producers in the UK occurred in 2001 involving *K. pneumoniae* with CTX-M-26 at one site, but by 2003, cloned and diverse *E. coli* with CTX-M-15 were widespread (Livermore and Hawkey, 2005). CTX-M-40 from an *E. coli* strain was reported in 2006 from the UK. The strain had been isolated from an immunocompromised hospital patient in 1998, which had initially been treated with piperacillin/tazobacam and netilmicin but did not respond clinically until piperacillin/tazobacam was replaced with meropenem (Hopkins et al., 2006).

Among 149 ESBL producing Enterobacteriaceae isolates collected from patients in Austria from 1998 to 2004, 38 *E. coli* isolates and 11 *Klebsiella* spp. were CTX-M producers. The proportion of CTX-M producers (group 1 and group 9) among all ESBL positive isolates rose from 0% in 1998 to 58% in 2004. One *E. coli* isolate was identical to the UK epidemic CTX-M-15- producing strain, although no epidemiological link with the UK was apparent (Eisner et al., 2006). Between January 2001 and April 2005 a large collection of human and animal isolates of *Salmonella* spp. was collected in Ireland to determine the prevalence of ESBLs. Seven ESBL producing isolates were detected. Two isolates produced CTX-M-15 and one isolate produced CTX-M-14 (Morris et al., 2006).

In 2005 a study was reported on the extent of ESBL producing Enterobacteriaceae at hospital and community level in Lebanon. Out of a total of 72 strains, 83% expressed the CTX-M-15 β-lactamase (Moubareck et al., 2005). The molecular epidemiology of ESBL producing *E. coli* isolates (n = 20) was investigated in a tertiary care-teaching hospital in Tel Aviv, Israel in 2005. 15 isolates exhibited CTX-M-2, and 3 isolates exhibited a new CTX-M-39 β-lactamase (Chmelnitsky et al., 2005). ESBL production was demonstrated in five independent, multi-drug-resistant isolates of enteroaggregative *E. coli* (EAEC) from the United Arab Emirates, representing 11.3% of the EAEC isolates recovered during 1 year. All five isolates carried the *bla*CTX-M-15. This is the first detailed description and characterization of ESBL production in EAEC and also the first report of CTX-M-producing organisms encountered on the Arabian Peninsula (Sonnevend et al., 2006).

Australia

The CTX-M-3 type ß-lactamase from a nosocomial *K. pneumoniae* isolate was reported for the first time in Australia by Paterson et al. in 2003.

Africa

The first report from Africa of a CTX-M-type β-lactamase (CTX-M-12) was from Kenya in 2001.This study involved nine *K. pneumoniae* isolates from new born babies at Kenyatta National Hospital in Nairobi, Kenya (Kariuki et al., 2001). CTX-M-15 reported in 2005 in Africa, was found in 5 *E. coli* isolates and 1 *K. pneumoniae* isolate out of 19 ESBL producing isolates in Tanzania. In this study only 1 *Salmonella* isolate was also ESBL positive, but did not produce the CTX-M enzyme (Blomberg et al., 2005). In 2005 Gangoue-Pieboji et al. reported the emergence of CTX-M-15 in three isolates of *K. pneumoniae* and *E. coli* derived from patients with urinary tract infections acquired during hospitalization in Cameroon. In Nigeria in 2005 ESBLs were characterized from 30 selected multi-drug resistant *K. pneumoniae* strains isolated from patients with community acquired urinary tract infections from Southwest Nigeria. The 30 strains produced multiple β-lactamases with 57% producing CTX-M β-lactamase. Only 2 CTX-M type genes were sequenced and were found to produce CTX-M-15 (Soge et al., 2006). The presence of β-lactamases with an extended spectrum of activity in 46 clinical *E. coli* isolates in Egypt was reported in 2006. 28 out of 46 strains produced CTX-M β-lactamases. CTX-M-14,-15 and -27 were found, with CTX-M-15 (25 out of the 28 strains) being the most prevalent (Al- Agamy et al., 2006). In 2001 a *Salmonella* isolate from the military hospital in Tunisia was found to produce a CTX-M-3 ESBL. CTX-M-27 production in 16 isolates of *S. enterica* serotype Livingstone were the cause of a nosocomial outbreak in the neonatal ward of Farhat Hached Hospital, in Tunisia in 2002 (Godet et al., 2005). In 2003 Paterson et al. identified a CTX-M-2-type and a CTX-M-3-type β-lactamases in 2 separate *K. pneumoniae* isolates from South Africa. This was the first report of CTX-M–type β-lactamases in South Africa. CTXM-37 was also reported in 2006 in Durban, South Africa from three *S. enterica* serotype Isangi strains. This study involved 59 putative ESBL *Salmonella* strains from a tertiary hospital in Durban (Govinden et al., 2006). A further study in 2006 of ESBL positive *Salmonella* sp. revealed the presence of a CTX-M-38 enzyme (Genbank accession number DQ864700). A study in Central African Republic was conducted between 2003 and 2005 to determine the fre-

quency of ESBLs and to characterize β-lactamases in 450 Enterobacteriaceae isolates at the Institut Pasteur de Bangui. Of the 4% of ESBL producing strains, CTX-M-15 was present in 10 *E. coli* and 1 *K. pneumoniae* isolate, whilst CTX-M-3 was present in only 1 *E. aerogenes* isolate (Frank et al., 2006). Four sequential ESBL-producing isolates of *K. pneumoniae* were detected during routine culture and susceptibility tests in the Ampath service laboratory in Cape Town, South Africa. The first and fourth isolates were susceptible to ertapenem, whereas the second and third were resistant. All 4 isolates belonged to the same strain and produced a group 1 CTX-M enzyme (Elliott et al., 2006).

BIOCHEMICAL EVOLUTION

Most CTX-M enzymes exhibit a much greater hydrolytic efficiency against cefotaxime than against ceftazidime. In the cefotaxime intermediate structure with Toho-1, residues Pro167, Asn170, Ser237, Asp240, and Arg274 are surrounded the bulky side chain of cefotaxime. In addition both side oxygens of Asp240 interact with the amino group in the aminothiazole ring, which may be involved in the binding of cefotaxime (Shimamura et al., 2002). In the CTX-M ESBLs, unlike those of TEM- and SHV-, increased activity against the bulky third generation cephalosporins, especially ceftazidime appears to occur not from gross enlargement of the active site, but from increased flexibility of the ß3 strand and possibly other regions. This increased flexibility is correlated with higher ceftazidimase activity and lower stability (Chen et al., 2005).

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. The Lys and Arg residues are positively charged and can form an electrostatic bond with the carboxylic acid group on oxyimino substituents of ceftazidime. Neutral residue Gly240 is not able to form electrostatic interactions with ß-lactams but could favor the accommodation of the oxyimino-ceftazidime side chain (Bonnet, 2004). Residue Gly240 is present in the ESBLs PER, VEB-1, and BES-1, which have hydrolytic activity against ceftazidime (Delmas et al., 2006).

Amino acid positions 240 and 167 seem to be involved in the evolution of CTX-M enzymes. CTX-M-15,-16,-27 and -32, which derive from CTX-M-3, -14,-9 and -1, respectively, by a Gly240Asp substitution, has greater catalytic efficiencies against ceftazidime (Bonnet, 2004). Munday et al. (2004) reported that CTX-M-25, which also has an Asp240Gly substitution, resulted in good enzymatic affinity towards ceftazidime, whilst CTX-M-26 which lacks the Asp240Gly substitution showed almost no activity towards ceftazidime. Comparison of the amino acid structures of other CTX-M enzymes available in the GenBank database reveals a glycine molecule at position 240 for CTX-M-28,-29,-33, -41 and -43, suggesting that these enzymes may also have ceftazidimase activity. To confirm the importance of Asp240-Gly substitution in the hydrolysis of ceftazidime, Cartelle et al. (2004) replaced the Gly240 with Asp in CTX-M-32 by using site directed mutagenesis. A lower MIC and lower catalytic efficiency was detected with the CTX-M-32 mutant. However site directed mutagenesis studies of CTX-M-9 by Aumeran and colleagues demonstrated that a substitution at position 240 of Asp240Lys (instead of Asp240Gly) was similar to mutations that promote ceftazidime activity found in the TEM and SHV ESBLs but did not result in increased hydrolysis of cetazidime for this enzyme (Munday et al., 2004). A random mutagenesis technique was used by Delmas et al. (2006) to predict the evolutionary potential of CTX-M-9 towards the acquisition of improved catalytic activity against ceftazidime. The mutants conferred 1- to 128- fold higher MICs of ceftazidime than the parental enzyme CTX-M-9. In addition to other mutants the substitutions Asp240Gly and Pro167Ser were noted. The kinetic constants of the three most active mutants revealed two distinct ways of improving catalytic efficiency against ceftazidime also suggesting that the CTX-M enzymes harbouring the substitution Asp240Gly are the most probable phylum for new mutants conferring the highest level of resistance to β-lactams (Delmas et al., 2006).

CTX-M-19, which derives from CTX-M-18 by a Pro167Ser substitution, is able to hydrolyze ceftazidime more efficiently than cefotaxime (Poirell et al., 2001). In laboratory-derived mutants of TEM-1, PSE-4 and BPS-1, a very similar mutation, Pro167Ser has been shown to be closely associated with ceftazidime resistance. CTX-M-23 with a Pro167Thr substitution is also associated with a higher level of resistance to ceftazidime than to cefotaxime. Even though residue 167 is not a direct part of the catalytic mechanism, this position seems to have a direct influence on substrate specificity (Stürenburg et al., 2004).

CONCLUSION

The widespread use of ceftriaxone and/or cefotaxime has been proposed as a reason for the emergence of CTX-M enzymes. The increased frequency of isolation and reporting of CTX-M ESBLs is alarming and is likely to represent only the tip of the iceberg for the under-developed continents where technology for the analysis of ESBL enzymes is scarce. The loss of the oxyimino-cephalosporins for treatment of infections represents a serious problem that seems to reach unprecedented levels globally (Munday et al., 2004). CTX-M enzymes are now endemic in many countries with both nosocomial and community emergence. The diversity of the CTX-M enzymes is noted especially in the Far East, Eastern Europe and Western Europe and some ESBL studies have identified CTX-M enzymes as the most prevalent

ESBL. The emergence of novel CTX-M β-lactamases in several countries is noted as opposed to the transfer of established CTX-M genes from one country to another, suggestive of a de novo dissemination of CTX-M genes. Despite many publications on ESBL enzymes, insight in the quantitative global distribution is lacking. A co-ordinated study to obtain this information is urgently needed.

REFERENCES

Abdalhamid B, Pitout DD, Moland ES, Hanson ND (2004). Community disease caused by *Citrobacter freundii* producing a novel CTX-M β-lactamase, CTX-M-30 in Canada. Antimicrob. Agents Chemother. 48: 4435-4437.

Ahmed AM, Nakano H, Shimamoto T (2004). The first characterization of extended-spectrum β-lactamase producing *Salmonella* in Japan. J. Antimicrob. Chemother. 57: 283-284.

Al-Agamy MH, Ashour ME, Wiegand I (2006). First description of CTX-M β-lactamase-producing clinical *Escherichia coli* isolates from Egypt. Int. J. Antimicrob. Agents 27: 545-548.

Bae IK, Lee BH, Hwang HY, Jeong SH, Hong SG, Chang CL, Kwak HS, Kim HJ, Youn H (2006). A novel ceftazidime-hydrolysing extended-spectrum β-lactamase, CTX-M-54, with a single amino acid substitution at position 167 in the omega loop. J. Antimicrob. Chemother. 58: 315-319.

Bonnet R, Sampaio JLM, Labia R, De Champs C, Sirot D, Chanal C, Sirot R (2000). A novel CTX-M β-lactamase (CTX-M-8) in cefotaxime-resistant Enterobacteriaceae isolated in Brazil. Antimicrob. Agents Chemother. 44: 1936-1942.

Bonnet R, Dutour C, Sampaio JLM, Chanal C, Sirot D, Labia R, De Champs C, Sirot J (2001). Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-240→Gly. Antimicrob. Agents Chemother. 45: 2269-2275.

Bonnet R (2004). Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. 48: 1-14.

Blomberg B, Jureen R, Manji KP, Tamin BS, Mwakagile DSM, Urassa WK, Fataki M, Msangi V, Tellevik MG, Maselle SY, Langeland N (2005). High rate of fatal cases of paediatric septicemia caused by gram-negative bacteria with extended-spectrum β-lactamases in Dar es Salaam, Tanzania. J. Clin. Microbiol. 43: 745-749.

Cartelle M, Thomas MD, Molina F, Moure R, Villaneuva R, Bou G (2004). High-level resistance to ceftazidime conferred by a novel enzyme, CTX-M-32, derived from CTX-M-1 through a single Asp240Gly substitution. Antimicrob. Agents Chemother. 48: 2308-2313.

Celenza G, Pellegrini C, Cacammo M, Segatore B, Amicosante G, Perilli P (2006). Spread of *bla* $_{\text{CTX-M-type}}$ and *bla*$_{\text{PER-2}}$ β-lactamase genes in clinical isolates from Bolivian hospitals. J. Antimicrob. Chemother. 57: 975-978.

Chen Y, Delmas J, Sirot J, Shoichet B, Bonnet R (2005). Atomic resolution structures of CTX-M β-lactamases: extended spectrum activities from increased mobility and decreased stability. J. Mol. Biol. 348: 349-362.

Chmelnitsky I, Carmeli Y, Leavitt A, Schwaber MJ, Navon-Venezia S (2005). CTX-M-2 and a new CTX-M-39 enzyme are the major extended-spectrum β-lactamases in multiple *Escherichia coli* clones isolated in Tel Aviv, Israel. Antimicrob. Agents Chemother. 49: 4745-4750.

Conceicao T, Brizio A, Duarte A, Lito LM, Cristino JM, Salgado MJ (2005). First description of CTX-M-15 producing *Klebsiella pneumoniae* in Portugal. Antimicrob. Agents Chemother. 49: 477-478.

Delmas J, Robin F, Carvalho F, Mongaret C, Bonnet R (2006). Prediction of the evolution of ceftazidime resistance in extended-spectrum β-lactamase CTX-M-9. Antimicrob. Agents Chemother. 50: 731-738.

Eckert C, Gautier V, Arlet G (2006). DNA sequence analysis of the genetic environment of various *bla*$_{\text{CTX-M}}$ genes. J. Antimicrob. Chemother. 57: 14-23.

Eisener A, Fagan EJ, Feierl G, Kessler HH, Marth E, Livermore DM, Woodford N (2006). Emergence of Enterobacteriaceae isolates producing CTX-M extended-spectrum β-lactamase in Austria. Antimicrob. Agents Chemother. 40: 785-787.

Elliot E, Brink AJ, Greune JV, Els Z, Woodford N, Turton J, Waner M, Livermore D (2006). *In vivo* development of ertapenem resistance in a patient with pneumoniae caused by *Klebsiella pneumoniae* with an extended-spectrum β-lactamase. Clin. Infect. Dis. 42: 95-98.

Frank T, Arlet G, Gautier V, Talarmin A, Bercion R (2006). Extended-spectrum β-lactamase-producing Enterobacteriaceae in Central African Republic. Emerg. Infect. Dis. 12: 863-864.

Godet OB, Salem YB, Fabre L, Demartin M, Grimont PAD, Mzoughi R, Weill FX (2005). Nosocomial outbreak caused by *Salmonella enterica* serotype Livingstone producing CTX-M-27 in a neonatal unit in Sousse, Tunisia. J. Clin. Microbiol. 43: 1037-1044.

Govinden U, Mocktar C, Moodley P, Sturm AW, Essack SY (2006). CTX-M-37 in *Salmonella enterica* serotype Isangi from Durban, South Africa. Int. J. Antimicrob. Agents 28: 288-291.

Hernandez JR, Martinez L, Canton R, Coque TM, Pascual A and the Spanish group for nosocomial infections (2005). Nationwide study of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases in Spain. Antimicrob. Agents Chemother. 49: 2122-2125.

Hopkins KL, Graham AD, Threlfall EJ (2006). Novel plasmid-mediated CTX-M-8 subgroup extended-spectrum β-lactamase (CTX-M-40) isolated in the UK. Int. J. Antimicrob. Agents 27: 570-575.

Ishii Y, Ohno A, Taguchi H, Imajo S, Ishiguro M, Matsuzawa H (1995).Cloning and sequence of the gene encoding a cefotaxime-hydrolyzing class A beta-lactamase isolated from *Escherichia coli* Antimicrob. Agents Chemother. 39: 2269-2275.

Kim J, Lim JM, Jeong YS, Seol SY (2005). Occurrence of CTX-M-3, CTX-M-15, CTX-M-14, and CTX-M-9 Extended-Spectrum ß-Lactamases in Enterobacteriaceae Clinical Isolates in Korea. Antimicrob. Agents Chemother. 49: 1572-1575.

Karim A, Poirel A, Nagarajan S, Nordman P (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.

Kariuki S, Corkill JE, Revathi G, Musoke R, Hart CA (2001) Molecular characterization of a novel plasmid-encoded cefotaximase (CTX-M-12) found in clinical *Klebsiella pneumoniae* isolates from Kenya. Antimicrob. Agents Chemother. 45: 2141-2143.

Kariuki S, Revathi G, Kariuki N, Muyodi J, Mwituria J, Munyalo A, Kagendo D, Murungi L, Hart CA (2005). Increasing prevalence of multidrug-resistant non-typhoidal salmonellae, Kenya, 1994-2003. Int. J. Antimicrob. Agents 25: 38-43.

Kim J, Lin YM, Jeong YS, Seol SY (2005). Occurrence of CTX-M-3, CTX-M-15, CTX-M-14 and CTX-M-9 extended-spectrum β-lactamases in Enterobacteriaceae clinical isolates in Korea, Antimicrob. Agents Chemother. 49: 1572-1575.

Koh TH, Wang GCY, Sng LH, Yi Z, Koh TY (2004). CTX-M and plasmid-mediated AmpC-producing Enterobacteriaceae, Singapore. Emerg. Infect. Dis. 10: 1172-1173.

Lartigue MF, Poirel L, Aubert D, Nordmann P (2006). In Vitro Analysis of IS*Ecp1B*-Mediated mobilization of naturally occurring ß-Lactamase gene *bla*$_{\text{CTX-M}}$ of *Kluyvera ascorbata.* Antimicrob. Agents Chemother. 50: 1282-1286.

Leflon-Guibout V, Jurand C, Bonacorsi S, Espinasse F, Guelfi MC, Duportail F, Heym B, Bingen E, Chanoin MHN (2004). Emergence and spread of three clonally related virulent isolates of CTX-M-15-producing *Escherichia coli* with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital. Antimicrob. Agents Chemother. 48: 3736-3742.

Liebana E, Batchelor M, Torres C, Brinas L, Lagos LA, Abdalhamid B, Hanson ND, Martinez-Urtaza JM (2004). Pediatric infection due to multiresistant *Salmonella enterica* serotype Infantis in Honduras. J. Clin. Microbiol. 42: 4885-4888.

Livermore DM, Hawkey M (2005). CTX-M: changing the face of ESBLs in the UK. J. Antimicrob. Chemother. 56:451-454.

Li H, Li JB (2005). Detection of five novel CTX-M-type extended-spectrum β-lactamases with one to three CTX-M-14 point mutations in isolates from Hefei, Anhui Province, China. J. Clin. Microbiol. 43:

4301-4302
Ma L, Ishii Y, Ishiguro M, Matsuzawa H, Yamaguchi K (1998). Cloning and sequencing of the gene encoding Toho-2, a class A β-lactamase preferentially inhibited by tazobactam. Antimicrob. Agents Chemother. 42: 1181-1186.
Mena A, Plasencia V, Gracia L, Ignacio J, Albert AS, Borrell N, Perez JL, Oliver A (2006). Characterization of a large outbreak by CTX-M-1-producing *Klebsiella pneumoniae* and mechanisms leading to *in vivo* carbapenem resistance development. J. Clin. Microbiol. 44: 2831-2837.
Miriagou V, Tassios PT, Legakis NJ, Tzouvelekis LS (2004). Expanded-spectrum cephalosporin resistace in non-typhoidal *Salmonella*. Int. J. Antimicrob. Agents 23: 547-555.
Morris D, Whelan M, Corbett-Feeney G, Cormican M, Hawkey P, Li X, Doran G (2006) First report of extended-spectrum β-lactamase-producing *Salmonella enterica* isolates in Ireland. Antimicrob. Agents Chemother. 50: 1608-1609.
Mugnaioli C, Luzzaro F, De Luca F, Brigante G, Amicosante G, Rossolini GM (2005). Dissemination of CTX-M-type extended-spectrum β-lactamase genes to unusual hosts. J. Clin. Microbiol. 42: 4183-4185.
Munday CJ, Boyd DA, Brenwald N, Miller M, Andrews JM, Wise R, Mulvey MR, Hawkey PM (2004). Molecular and kinetic comparison of the novel extended-spectrum β-lactamases CTX-M-25 and CTX-M-26. Antimicrob. Agents Chemother. 48: 4829-4834.
Moubareck C, Daoud Z, Hakime NI, Hamze M, Mangeney N, Matta H, Mokhbat E, Raymond R, Sarkis DK, Populaire FD (2005). Countrywide spread of community- and hospital-acquired extended-spectrum β-lactamase (CTX-M-15) producing Enterobactericeae in Lebanon. J. Clin. Microbiol. 43: 3309-3313.
Naiemi NA, Bart A, De Jong MD, Vandenbroucke-Gruls C.M, Rietra PJGM, Depets-Ossenkopp YJ, Wever PC, Spanjaard L, Bos AJ, Dium B. (2006). Widely distributed and predominant CTX-M extended-spectrum β-lactamases in Amsterdam, the Netherlands. J. Clin. Microbiol. 43: 3012-3014.
Pagani L, Dell' Amico E, Migliavacca R, D'Andrea MM, Giacobone E, Amicosante G, Romero E, Rossolini GM (2003). Multiple CTX-M-type extended-spectrum β-lactamases in nosocomial isolates of Enterobacteriaceae from a hospital in northern Italy. J. Clin. Microbiol. 41: 4264-4269.
Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, Bonomo RA and the international *Klebsiella* study group (2003). Extended-spectrum β-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries. Dominance and widespread prevalence of SHV- and CTX-M-type β-lactamases. Antimicrob. Agents Chemother. 47: 3554-3564.
Gangoué-Piéboji J, Bedenic B, Shiro SK, Randegger C, Adiogo D, Ngassam P, Ndumbe P, Hachler H (2005). Extended-spectrum β-lactamase producing Enterobacteriaceae in Yaounde Cameroon. J. Clin. Microbiol. 43: 3273-3277.
Pitout JDD, Nordman P, Laupland KB, Poirel L (2005 a). Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases in the community. J. Antimicrob. Chemother. 56: 52-59.
Pitout JDD, Gregson DB, Church DL, Elsayed S, Laupland KB (2005b). Community-wide outbreaks of clonally related CTX-M-14 β-lactamase-producing *Escherichia coli* strains in the Calgary health region. J. Clin. Microbiol. 43: 2844-2849.
Poirel L, Naas T, Thomas I, Karim A, Bingen E, Nordman P (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.
Pottumarthy S, Yu Y, Sader HS, Jones RN, Chen M (2005). Susceptibility testing accuracy of a CTX-M-type extended-spectrum β-lactamase organism-producing population of Enterobacteriaceae: intermethod analysis for 9 β-lactams. Diag. Microbiol. Infect. Dis. 53: 131-141.
Quinteros M, Radice M, Gardella N, Rodriguez M, Costa N, Korbenfeld D, Couto E, Gutkind G, and the microbiology study group (2003). Extended-spectrum β-lactamases in Enterobacteriaceae in Buenos Aires, Argentina, public hospitals. Antimicrob. Agents Chemother. 47: 2864-2867.
Rasmussen JW, Hoiby N (2004). Cefotaximases (CTX-M-ases), an expanding family of extended-spectrum β-lactamases. Can. J. Microbiol. 50: 137-165
Shibata N, Kurokawa H, Doi Y, Yagi T, Yamane K, Wachino JI, Suzuki S, Kimura K, Ishikawa S, Kato H, Ozawa Y, Shibayama K, Kai K, Konda T, Arakawa Y (2006). PCR classification of CTX-M-type β-lactamase genes identified in clinically isolated gram-negative bacilli in Japan. Antimicrob. Agents Chemother. 50: 791-795.
Shimamura T, Ibuka A, Fushinobu S, Wakagi T, Ishiguro M, Ishii Y, Matsuzawa H (2002). Acyl-intermediate structures of the extended-spectrum class A β-lactamase, Toho-1 in complex with cefotaxime, cephalothin and benzylpenicillin. J. Biol. Chem. 277: 46601-46608.
Simarro E, Navarro F, Ruiz J, Miro E, Gomez J, Mirelis B (2000). *Salmonella enterica* serovar Virchow with CTX-M-like β-lactamase in Spain. J. Clin. Microbiol. 38: 4676-4678.
Soge OO, Queenan AM, Ojo KK, Adeniyi BA, Roberts MC (2006). CTX-M-15 extended spectrum β-lactamase from Nigerian *Klebsiella pneumoniae*. J. Animicrob. Chemother. 57: 24-30.
Sonnevend A, Dhaheri KA, Mag T, Herpay M, Kolodziejek J, Noworthy N, Usmani A, Sheikh FA, Pai T (2006). CTX-M-15-producing multidrug-resistant enteroaggregative *Escherichia coli* in the United Arab Emirates. Clin. Microbiol. Infect. 12: 582.
Stürenburg E, Mack D (2003). Extended-spectrum β-lactamases: implications for the clinical microbioloby laboratory, therapy and infection control. J. Infect. 47: 273-295
Stürenburg E, Kuhn A, Mack D, Laufs R (2004). A novel extended-spectrum β-lactamase CTX-M-23 with a P167T substitution in the active-site omega loop associated with ceftazidime resistance. J. Antimicrob. Chemother. 54:406-409.
Tzouvelekis LS, Tzelepi E, Tassios PT, Legakis NJ (2000). CTX-M-type β-lactamases: an emerging group of extended -spectrum enzymes. Int. J. Antimicrob. Agents 14: 137-142.
Villegas MV, Correa A, Perez F, Zuluaga T, Radice M, Gutkind G, Casellas JM, Ayala J, Lolans K, Quinn JP and the Colombian nosocomial resistance study group (2004). CTX-M-12 β-lactamase in a *Klebsiella pneumoniae* clinical isolate in Colombia. Antimicrob. Agents Chemother. 48: 629-631.
Wu TL, Chia JH, Su LH, Kuo AJ, Chu C, Chiu CH (2003). Dissemination of extended-spectrum β-lactamase-producing Enterobacteriaceae in paediatric intensive care units J. Clin. Microbiol. 41: 4836-4838
Yan JJ, Hsueh PR, Lu JJ, Chang FY, Shyr JM, Wan JH, Liu YC, Chuang YC, Yang YC, Tsao SM, Wu HH, Wang LS, Lin TP, Wu HM, Chen HM, Wu JJ (2006). Extended-spectrum β-lactamases among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from seven medical centres in Taiwan. Antimicrob. Agents Chemother. 50: 1861-1864.
Yong D, Lim YS, Yum JH, Lee H, Lee K, Kim EC, Lee BK, Chong Y (2005). Nosocomial outbreak of pediatric gastroenteritis cause by CTX-M-14 type extended-spectrum β-lactamase-producing strains of *Salmonella enterica* serovar London. J. Clin. Microbiol. 43: 3519-3521.