Yonsei Medical Journal
Vol. 39, No. 6, pp. 520-525, 1998

☐ Review Article ☐

Plasmid-Encoded AmpC β-Lactamases: How Far Have We Gone 10 Years after the Discovery?

Adolf Bauernfeind[1], Yunsop Chong[2], and Kyungwon Lee[2]

The dogma that ampC *genes are located exclusively on the chromosome was dominant until about 10 years ago. Since 1989 over 15 different plasmid-encoded AmpC β-lactamases have been reported from several countries. Most of these enzymes evolved in two clusters. The major cluster includes several enzymes with a high similarity to CMY-2, which is the closest related chromosomal AmpC enzyme of* Citrobacter freundii. *A second cluster centers around CMY-1. It is less homogeneous and not closely related chromosomal AmpC enzymes. Molecular diversification by amino acid substitutions does not usually translate into a change in the resistance phenotype. At this time, CMY-2 appears to be the most prevalent and widely distributed. Further global increase of prevalence and diversity of plasmidic AmpC β-lactamases have to be anticipated in the next millenium.*

Key Words: AmpC β-lactamases, plasmid-encoded β-lactamases, CMY-1, CMY-2, molecular evolution of β-lactamases

Among gram-negative bacteria, enzymatic resistance to β-lactams is mediated predominantly by two major types of β-lactamases; chromosomally-encoded enzymes produced in high quantity either constitutively or after induction (Ambler class C, e.g. in *Citrobacter* (Lindberg and Normark 1986), *Enterobacter* (Galleni *et al.* 1988), *Serratia* (Nomura and Yoshida, 1990), *Morganella morganii* (Barnaud *et al.* 1997), *Yersinia enterocolitica* (Seoane *et al.* 1992), *Pseudomonas aeruginosa* (Lodge *et al.* 1990) or plasmid-encoded enzymes of Ambler class A detected mainly in *Escherichia coli, Klebsiella pneumoniae, Salmonella* spp., and *Shigella* spp. These species usually do not produce AmpC β-lactamases in amounts high enough to reach clinical resistance. The dogma that *ampC* genes are located exclusively on the chromosome was dominant until about 10 years ago. At that time two novel plasmidic *bla* genes emerged independently at two widely distant parts of the world, namely in Providence, R.I., USA (Papanicolau *et al.* 1990) and in Seoul, South Korea (Bauernfeind *et al.* 1989). For the first time AmpC-type *bla* genes were found to reside on transmissible plasmids. There was no apparent link between these two events and the nucleotide sequence of the genes coding for the enzymes are highly dissimilar.

Were these discoveries just episodic curiosities rising and setting at two locations more or less simultaneously by chance? In fact, these reports heralded a new evolutionary line in the development of antibiotic resistance in human pathogens. Looking back over the past decade tells us that we witnessed the dawn of a group of β-lactamases which have since become a clinically relevant factor of antibiotic resistance.

Received October 15, 1998
[1]Max von Pettenkofer Institut für Hygiene und Medizinische Mikrobiologie der Ludwig-Maximilians-Universitat München, München, Germany, [2]Department of Clinical Pathology and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea

Address reprint request to Dr. A. Bauernfeind, Max von Pettenkofer Institut Pettenkoferstr. 9a, D-80336 München, Germany. Tel: 49-89-5160-5268, Fax: 49-89-5160-5266, E-mail: Adolf.Bauernfeind@mvp-bak.med.uni-muenchen.de

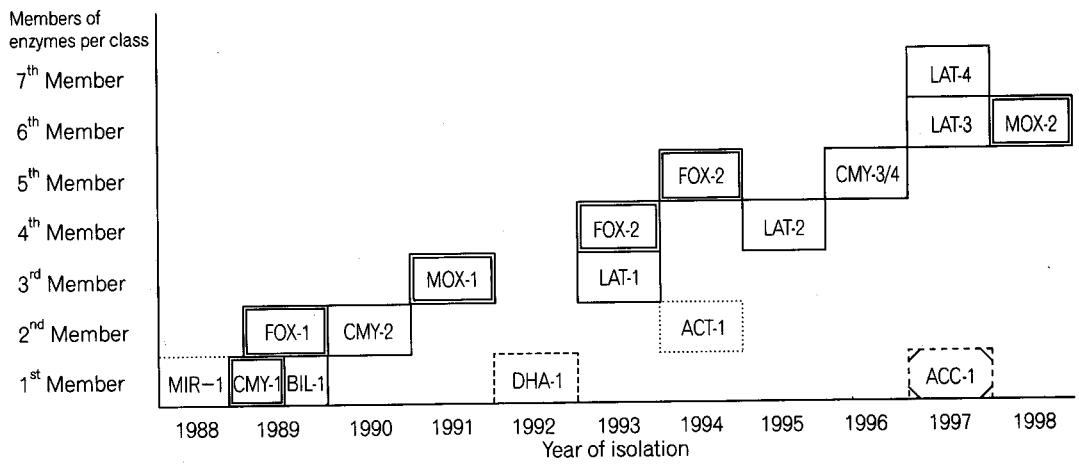

Fig. 1. *Chronology of detection and classification of plasmid-encoded AmpC β-lactamases. Since the late 1980s, the number of plasmid-encoded AmpC β-lactamases has been increasing continuously by 1 to 2 new enzymes per year.* ⬚ *MIR-1/ACT-1 family,* ▣ *CMY-1/FOX/MOX family,* □ *BIL-1/CMY-2/LAT family,* ⬚ *DHA-1,* ⬚ *ACC-1.*

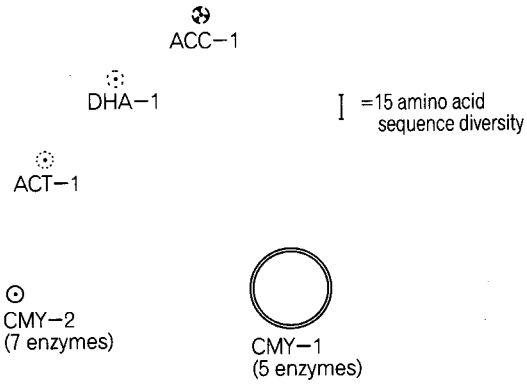

Fig. 2. *The 1998 cosmos of plasmidic AmpC-genes. Polycentric constellation: one seven-membered group of closely related enzymes (CMY-2,-3/4; LAT-1,-2,-3,-4; BIL-1), one five-membered group of moderately related enzymes (CMY-1; MOX-1; FOX-1,-2,-3) and three solitary types. The distances are proportional to the number of amino acid sequence differences.*

Chronology of identification and molecular relationship of plasmid-encoded AmpC β-lactamases

Since the late 1980s, the number of plasmid-encoded AmpC β-lactamases has been increasing continuously by 1 to 2 (1.5 on average) new enzymes per year (Fig. 1). The classification of β-lactamases may rely on phenotypic or genotypic characters. The presumed amino acid sequence obtained by translation of the nucleotide sequence is used for genetic classification. It determines the molecular fingerprint of the individual enzyme and allows the analysis of relationships among β-lactamases (Table 1).

Looking at the emergence of AmpC-type β-lactamases over time, their evolution appears to proceed polycentrically around several ancestor enzymes (Fig. 2). At least two clusters of closely related enzymes may be separated. The major cluster includes enzymes with a high similarity to CMY-2 (Bauernfeind *et al.* 1996) (between 99.7% and 97.9% identity, that is 1 to 8 amino acids different from CMY-2). The closest related (95.8% identity, 16 amino acid substitutions) chromosomal AmpC enzyme is that of *C. freundii.*

Another cluster is associated to CMY-1. It is less homogeneous and includes enzymes with amino acid identities between 96.9% and 89.5% (11 to 38 amino acid exchanges). There is no closely related chromosomal AmpC enzyme, since the least distant is AmpC of *Pseudomonas aeruginosa* which demonstrates only 54.4% identity in amino acid sequence with CMY-1.

Table 1. Percentage of amino acid identity between AmpC β-lactamases

β-lactamase and species*	Cfr	Ecl	Eco	Mmo	Pae	Sma	Yen	CMY-2	CMY-3/4	LAT-1	LAT-2	LAT-3	LAT-4	BIL-1	CMY-1	MOX-1	FOX-1	FOX-2	FOX-3	DHA-1	ACT-1	ACC-1
AmpC Cfr	100	73.3	76.7	57.2	42.3	38.2	56.2	95.8	95.5	95.0	95.5	94.0	95.5	94.2	41.3	37.4	42.7	42.4	41.6	57.5	73.9	40.0
AmpC Ecl		100	69.8	54.8	44.7	41.2	54.9	75.1	74.8	74.3	74.8	73.2	74.8	73.5	43.5	39.8	44.0	44.0	43.2	55.3	87.6	37.1
AmpC Eco			100	58.2	43.0	39.1	58.1	77.7	77.4	77.2	77.4	75.9	77.4	76.7	41.1	37.2	42.2	42.2	41.6	58.7	71.8	39.0
AmpC Mmo				100	48.1	40.3	52.5	58.0	58.0	57.5	58.3	56.7	57.5	57.7	43.6	39.9	46.4	46.1	46.7	98.9	56.3	37.0
AmpC Pae					100	44.9	43.0	42.3	42.3	42.1	42.3	41.5	41.8	42.1	54.4	49.5	53.7	53.7	53.4	48.4	45.8	43.5
AmpC Sma						100	38.7	38.2	38.2	37.9	37.9	37.1	37.7	37.4	44.5	40.6	45.6	45.6	45.0	40.7	39.6	52.3
AmpC Yen							100	56.2	55.9	55.6	56.2	54.8	55.4	55.4	40.6	36.0	42.8	42.8	42.2	53.0	54.8	38.9
CMY-2 Kpn								100	99.7	98.9	99.5	97.9	98.9	98.4	41.3	37.7	42.9	42.7	41.8	58.0	75.3	39.8
CMY-3/4 Pmi									100	98.7	99.2	97.9	98.7	98.2	41.3	37.7	42.9	42.7	41.8	58.0	75.0	39.5
LAT-1 Kpn										100	98.9	98.2	98.9	97.4	41.0	37.4	42.7	42.4	41.6	57.4	74.5	39.5
LAT-2 Kpn											100	97.9	98.9	97.9	41.0	37.4	42.9	42.7	41.8	58.2	75.0	39.5
LAT-3 Eco												100	98.4	96.3	40.5	37.7	42.1	41.8	41.0	56.6	74.4	39.0
LAT-4 Eco													100	97.4	41.0	37.7	42.7	42.4	41.6	57.4	75.0	39.0
BIL-1 Eco														100	40.5	36.9	42.1	41.8	41.0	57.7	73.9	39.0
CMY-1 Kpn															100	89.5	73.6	74.9	73.1	43.6	41.2	43.9
MOX-1 Kpn																100	66.7	67.3	66.1	39.9	38.2	40.8
FOX-1 Kpn																	100	96.9	95.0	46.6	42.0	45.0
FOX-2 Eco																		100	96.3	46.2	42.0	46.4
FOX-3 Kox																			100	46.7	41.5	45.5
DHA-1 Sal																				100	56.3	37.0
ACT-1 Kpn																					100	38.2
ACC-1 Kpn																						100

*: Cfr, *Citrobacter freundii*; Ecl, *Enterobacter cloacae*; Eco, *Escherichia coli*; Mmo, *Morganella morganii*; Pae, *Pseudomonas aeruginosa*; Sma, *Serratia marcescens*; Yen, *Yersinia enterocolitica*; Kpn, *Klebsiella pneumoniae*; Pmi, *Proteus mirabilis*; Kox, *Klebsiella oxytoca*; Sal, *Salmonella enteritidis*.

Table 2. Amino acid exchanges within the CMY-2 family

Type of β-lactamase	Amino acid at position																
	S11	75	77	78	100	139	193	201	202	215	289	296	319	338	339	348	352
CMY-2	C	G	A	I	Q	Q	Q	W	G	Q	S	L	T	M	L	V	A
CMY-3/4								R									
LAT-2			C				E										
LAT-4			C							R			S			A	
LAT-1	S		C		K					R							
LAT-3			C	M	E	L		L		R			S				G
BIL-1		R							A		T	V		I	V		

The number of amino acid substitutions indicates that a direct phylogenetic lineage may exist within the CMY-2 cluster. The evolution may have proceeded in the following sequence: CMY-2 → CMY-3/-4 (Bret *et al.* 1998) → LAT-2 (Gazouli *et al.* 1996) → LAT-1 (Tzouvelekis *et al.* 1993), LAT-4 (Gazouli *et al.* 1998) → LAT-3 (Gazouli *et al.* 1998) → BIL-1 (Fosberry *et al.* 1994). In comparison with CMY-2, amino acid substitutions are found in 16 positions altogether (plus 1 exchange in the signal peptide) (Table 2). The number of amino acid substitutions is between 1 (CMY-3/-4) and 8 (LAT-3). By this procedure, enzyme-specific fingerprints within the CMY-2 cluster are demonstrated. There was no exchange close to the active site serine at position 64. The closest substitution was found at a distance of 11 amino acids at position 75. As no major differences in resistance phenotype between the enzymes were described, it may be supposed that these 16 sites do not have a pronounced effect on the catalytic activity.

Of the remaining enzymes, DHA-1 (Barnaud *et al.* 1998) and ACT-1 (Bradford *et al.* 1997) demonstrate a low percentage of amino acid identity (56.3%) between one another, DHA-1 is closely related to AmpC of *M. morganii* (98.9%), while ACT-1 is most similar to AmpC of *E. cloacae* (87.6%). The recently detected AmpC-type β-lactamase ACC-1 (Bauernfeind *et al.* 1998) is the most unique of all. Its maximum amino acid sequence identity is 52.3% (AmpC of *Serratia marcescens*) or 46.4% (FOX-2, Bauernfeind *et al.* 1997b).

Relationship between structure and function of plasmid-encoded AmpC β-lactamases

The process of molecular diversification is traceable by sequencing of the *bla*-genes. Molecular diversification by amino acid substitutions does not usually translate into a change in the resistance phenotype. To disclose relationships between structure and function in AmpC β-lactamases, strains with unusual resistance phenotype are useful as shown for CMY-1. This enzyme was first detected in 1989 in Seoul. In 1995/96, the β-lactamase was still present in isolates from patients in the same area. The strains demonstrated an identical resistance phenotype, except for MICs of ceftazidime which was 4 μg/ml in 1989 and 64 μg/ml in 1995. This finding opened an opportunity to investigate the structure-activity relationship. So the *bla*-gene of the strains from 1995 was sequenced and compared with the sequence of the 1989 strain. Surprisingly, 99.7% identity in the amino acid sequence was found, and the divergence was restricted to a single amino acid exchange, namely from Asn at position 346 to Ile. When the Ile^{346} was substituted with site-directed mutagenesis by Asn again, as in the original strain, susceptibility to ceftazidime was reconstituted (Bauernfeind *et al.* 1997a). This indicated a catalytic function of position 346 in the structure of AmpC enzymes.

Further extension of the knowledge about the structure-activity relationship in AmpC enzymes may be expected from in vitro amino acid substitutions - a rather laborious procedure, difficult to focus by selection both of promising sites and amino acids for exchange - or from clinical isolates ex-

pressing an unusual resistance phenotype. Though the second approach is easier to analyse and to interpret, its yield relies on the accidental detection of mutants.

Distribution of plasmid-encoded AmpC β-lactamases

Plasmid-encoded AmpC β-lactamases were found to be produced by numerous pathogens, e.g. *K. pneumoniae, E. coli, Salmonella* spp., *P. mirabilis,* and *C. freundii*. Meanwhile, they were detected in many countries as compiled in Table 3. At this time, CMY-2 appears to be the most frequent and widely distributed type of plasmid-mediated AmpC β-lactamase.

There is only a rather limited amount of information from which predictions for further development may be deduced. So far, β-lactamases dissimilar enough from known ones to justify their nomination as new enzymes have been shown to evolve to multi-membered families of related enzymes. The number of amino acid differences may be low enough to assume a linear evolution between related enzymes (e.g. within the CMY-2 group). A tentative calibration of the phylogenetic clock might be the time period between CMY-1a (1989) and CMY-1b (1995). Assuming that CMY-1b did not appear before 1995, it would have taken 6 years for one point mutation. In conclusion we have to anticipate that the evolution of *ampC* genes will continue as the antibiotic selective pressure will persist. This will result in further global increase of prevalence and diversity of plasmidic AmpC β-lactamases in the next millenium.

Table 3. Global distribution of plasmid-encoded AmpC β-lactamase

Type of β-lactamase	Detected in
MIR-1 (Papanicolaou *et al.* 1990)	USA
CMY-1 (Bauernfeind *et al.* 1989)	Korea
BIL-1 (Fosberry *et al.* 1994)	Pakistan
FOX-1 (Gonzalez Leiza *et al.* 1994)	Argentina
CMY-2 (Bauernfeind *et al.* 1996)	Greece, Turkey, Germany, USA
MOX-1 (Horii *et al.* 1994)	Japan
DHA-1 (Barnaud *et al.* 1998)	Saudi Arabia
LAT-1 (Tzouvelekis *et al.* 1993)	Greece
FOX-2 (Bauernfeind *et al.* 1997b)	Guatemala
ACT-1 (Bradford *et al.* 1997)	USA
FOX-3 (Marchese *et al.* 1998)	Italy
LAT-2 (Gazouli *et al.* 1996)	Greece
CMY-3/-4 (Bret *et al.* 1998)	France, Tunisia
ACC-1 (Bauernfeind *et al.* 1998)	Germany
LAT-3 (Gazouli *et al.* 1998)	Greece
LAT-4 (Gazouli *et al.* 1998)	Greece
MOX-2 (Boyer-Mariotte *et al.* 1998)	Greece

REFERENCES

Barnaud G, Arlet G, Danglot C, Philippon A: Cloning and sequencing of the gene encoding the AmpC β-lactamase of *Morganella morganii. FEMS Microbiol Lett 148: 15-20, 1997*

Barnaud G, Arlet G, Verdet C, Gaillot O, Lagrange PH, Philippon A: *Salmonella enteritidis*: AmpC plasmid-mediated inducible β-lactamase (DHA-1) with an *ampR* gene from *Morganella morganii. Antimicrob Agents Chemother 42: 2352-2358, 1998*

Bauernfeind A, Chong Y, Schweighart S: Extended broad spectrum β-lactamase in *Klebsiella pneumoniae* including resistance to cephamycins. *Infection 17: 316-321, 1989*

Bauernfeind A, Schneider I, Jungwirth R, Sahly H, Ullmann U: A novel type of AmpC β-lactamase, ACC-1, produced by a *Klebsiella pneumoniae* causing nosocomial pneumonia. *Antimicrob Agents Chemother* (submitted), 1998

Bauernfeind A, Schneider I, Meyer D, Jungwirth R, Chong Y, Lee K: Possible role of amino acid substitution at position 346 on the ceftazidime-hydrolyzing activity of the cephamycinase CMY-1, abstr. C203. In Abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington D.C., American Society for Microbiology, 1997a, 80

Bauernfeind A, Stemplinger I, Jungwirth R, Giamarellou H: Characterization of the plasmidic β-lactamase CMY-2, which is responsible for cephamycin resistance. *Antimicrob Agents Chemother 40: 221-224, 1996*

Bauernfeind A, Wagner S, Jungwirth R, Schneider I, Meyer D: A novel class C β-lactamase (FOX-2) in *Escherichia coli* conferring resistance to cephamycins. *Antimicrob Agents Chemother 41: 2041-2046, 1997b*

Boyer-Mariotte S, Raskine L, Hanau B, Philippon A, Sanson-Le Pors MJ, Arlet G: Novel plasmid-mediated AmpC-type β-lactamase (MOX-2) in a clinical isolate of *Klebsiella pneumoniae*, abstr. C7.

In Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington D.C., American Society for Microbiology, 1998, 70

Bradford PA, Urban C, Mariano N, Projan SJ, Rahal JJ, Bush K: Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC β-lactamase, and the loss of an outer membrane protein. *Antimicrob Agents Chemother 41: 563-569, 1997*

Bret L, Chanal-Claris C, Sirot D, Chaibi EB, Labia R, Sirot J: Chromosomally encoded AmpC-type β-lactamase in a clinical isolates of *Proteus mirabilis*. *Antimicrob Agents Chemother 42: 1110-1114, 1998*

Fosberry AP, Payne DJ, Lowlor EJ, Hodgson JE: Cloning and sequencing analysis of bla_{BIL-1}, a plasmid-mediated class C β-lactamase gene in *Escherichia coli* BS. *Antimicrob Agents Chemother 38: 1182-1185, 1994*

Galleni M, Lindberg F, Normark S, Cole S, Honore N, Joris B, Frere J-M: Sequence analysis of three *Enterobacter cloacae ampC* β-lactamase genes and their products. *J Biochem 250: 753-760, 1988*

Gazouli M, Tzouvelekis LS, Prinarakis E, Miriagou V, Tzelepi E: Transferable cefoxitin resistance in enterobacteria from Greek hospitals and characterization of a plasmid-mediated group 1 β-lactamase (LAT-2). *Antimicrob Agents Chemother 40: 1736-1740, 1996*

Gazouli M, Tzouvelekis LS, Vatopoulos AC, Tzelepi E: Transferable class C β-lactamase in *Escherichia coli* strains isolated in Greek hospitals and characterization of two enzyme variants (LAT-3 and LAT-4) closely related to *Citrobacter freundii* AmpC β-lactamase. *J Antimicrob Chemother* (submitted), 1998

Gonzalez Leiza M, Perez-Diaz JC, Ayala J, Casellas JM, Martinez-Beltran J, Bush K, Baquero F: Gene sequence and biochemical characterization of FOX-1 from *Klebsiella pneumoniae*, a new AmpC-type plasmid-mediated β-lactamase with two molecular variants. *Antimicrob Agents Chemother 38: 2150-2157, 1994*

Horii T, Arakawa Y, Ohta M, Sugiyama T, Wacharotayankum R, Ito H, Kato N: Characterization of a plasmid-borne and constitutively expressed bla_{MOX-1} gene encoding AmpC-type β-lactamase. *Gene 139: 93-98, 1994*

Lindberg F, Normark S: Sequence of the *Citrobacter freundii* OS60 chromosomal *ampC* β-lactamase gene. *Eur J Biochem 156: 441-445, 1986*

Lodgé JM, Minchin SD, Piddock LJV, Busby SJW: Cloning, sequencing and analysis of the structural gene and regulatory region of the *Pseudomonas aeruginosa* chromosomal AmpC β-lactamase. *Biochem J 272: 627-631, 1990*

Marchese A, Arlet G, Schito GC, Lagrange PH, Philippon A: Characterization of FOX-3, an AmpC-type plasmid-mediated β-lactamase from an Italian isolate of *Klebsiella oxytoca. Antimicrob Agents Chemother 42: 464-467, 1998*

Nomura K, Yoshida T: Nucleotide sequence of the *Serratia marcescens* SR50 chromosomal *ampC* β-lactamase gene. *FEMS Microbiol Lett 70: 295-300, 1990*

Papanicolau GA, Medeiros AA, Jacoby GA: Novel plasmid-mediated β-lactamase (MIR-1) conferring resistance to oxyimino- and α-methoxy β-lactams in clinical isolates of *Klebsiella pneumoniae. Antimicrob Agents Chemother 34: 2200-2209, 1990*

Seoane A, Francia MV, Garcia Lobo JM: Nucleotide sequence of the *ampC-ampR* region from the chromosome of *Yersinia enterocolitica. Antimicrob Agents Chemother 36: 1049-1052, 1992*

Tzouvelekis LS, Tzelepi E, Mentis AF, Tsakaris A: Identification of a novel plasmid-mediated β-lactamase with chromosomal cephalosporinase characteristics from *Klebsiella pneumoniae. J Antimicrob Chemother 31: 645-654, 1993*