Eur. J. Clin. Microbiol. Infect. Dis. 1994, Supplement 1, p. 17–29
0934-9723/94/Suppl. 1 0017-13 $ 3.00/0

Origin and Impact of Plasmid-Mediated Extended-Spectrum Beta-Lactamases

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Resistance to oxyimino cephalosporins was originally highlighted by the emergence of plasmid-encoded extended-spectrum β-lactamases deriving by mutation from TEM-1, TEM-2 and SHV type enzymes (class A). The broader spectrum of resistance produced by these enzymes is related to more amino acid substitutions, but susceptibility to seven alpha-methoxyimino cephalosporins and carbapenems was preserved until recently. Clavulanate-sensitive extended-spectrum β-lactamases are distributed worldwide, mainly among *Klebsiella pneumoniae* isolates. Novel clavulanate-sensitive extended-spectrum β-lactamases deriving from other class A enzymes (e.g. MEN-1 from βla OXY, OXA-11 in *Pseudomonas aeruginosa* from PSE-2) have been reported. Recently, clavulanate-resistant extended-spectrum β-lactamases (class C) were encountered amongst single isolates, mostly *Klebsiella pneumoniae*. These cephalosporinases or cefamycinases (usually chromosomally mediated) have expanded the spectrum of plasmid-encoded resistance to include seven alpha-methoxyimino cephalosporins. Thus far, only two isolates (1 *Pseudomonas aeruginosa*, 1 *Bacteroides fragilis*), both recovered in Japan, with plasmid-mediated resistance to carbapenems have been found.

The major mechanism of resistance to β-lactam antibiotics among clinical gram-negative isolates is related to the production of β-lactamase. Many β-lactams, including extended broad-spectrum cephalosporins, which tolerate the β-lactamases, have been improved for clinical purposes.

Nevertheless, the development of these highly stable extended-spectrum cephalosporins at the beginning of the 1980s was quickly followed by the emergence of several transmissible extended-spectrum β-lactamases identified among nosocomial isolates of *Klebsiella pneumoniae* (review in 1, 2). Such β-lactamases became problematic clinically and have been shown to be derived from SHV or TEM type β-lactamases by one or more amino acid substitutions (2). These β-lactamases effectively hydrolyze broad-spectrum β-lactam antibiotics such as penicillins and cephalosporins, including oxyimino β-lactams (cefotaxime, ceftazidime, aztreonam). Fortunately, they do not affect cefamycins or methoxyimino cephalosporins, carbapenems or penems.

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More recently, several plasmid-mediated so-called extended-spectrum β-lactamases (FEC-1, MEN-1, MIR-1, CMY-1, CMY-2, CMY-M, BIL-1, MOX-1, LAT-1 and OXA-11) were reported in several countries (3–11, A. Bauernfeind et al., 30th ICAAC, Atlanta, 1990, Abstract no. 190). Some of them exhibited a wider spectrum of resistance including β-lactamase inhibitors and methoxyimino β-lactams (cefoxitin, cefotetan, moxalactam). Thus far, plasmid-encoded enzymatic resistance to carbapenems has been rare, reported in only two clinical strains (*Pseudomonas aeruginosa*, *Bacteroides fragilis*) (12, 13).

In view of the obvious differences between enzymes classified as extended-spectrum β-lactamases, i.e. those producing resistance at least to oxyimino β-lactams, there is a need to clearly define these enzymes, which have received several diverse denominations (cefotaximase, ceftazidimase, extended broad-spectrum β-lactamase, methoxyimino β-lactamase, cefamycinase and oxyimino cephalosporinase) (2, 9, 11). In fact, the original definition of extended-spectrum β-lactamases referred to all plasmid-mediated enzymes derived from TEM and SHV types and causing resistance to extended-spectrum cephalosporins (1). Nevertheless, two other definitions emerged

in the literature designating as extended-spectrum β-lactamases all plasmid-encoded β-lactamases that hydrolyze oxyimino β-lactams (2) or those enzymes that hydrolyze extended broad-spectrum β-lactams and are strongly inhibited by clavulanate (14). The former definition includes the chromosomal β-lactamase produced, even at low levels, by *Klebsiella oxytoca* strains. Differentiation of these enzymes is essential because their spectrum of inactivation and their magnitude differ according to the group examined.

Keeping in mind the Ambler classification of β-lactamases with class A (penicillinases, broad-spectrum enzymes), class B (metalloenzymes), class C (cephalosporinases) and class D (some oxacillin-hydrolyzing enzymes) (15, 16), it seems justified to classify the plasmid-mediated extended-spectrum β-lactamases according to the following scheme: class A, including TEM-3 to TEM-26 and SHV-2 to SHV-6; class B, with two undesignated metalloenzymes (MET); class C, with MIR-1, BIL-1, CMY-2, MOX-1 and LAT-1; and class D, with one novel example (OXA-11). Some other extended-spectrum enzymes such as FEC-1, FPM-1, CMY-1 and CTX-M cannot be placed into such defined classes in the absence of results relative to biochemical properties, amino acid or DNA sequence, and DNA hybridization.

According to their main properties, the plasmid-mediated extended-spectrum β-lactamases can be divided in several groups, as outlined below.

Clavulanate-Sensitive TEM and SHV Type Beta-Lactamases (Class A)

The majority of the plasmid-encoded β-lactamases belong to class A and have been grouped as TEM- or SHV-derived β-lactamases on the basis of their substrate and inhibition profiles, isoelectric points, DNA hybridization and amino acid sequences (1, 2, 17, 18). At least 25 different enzymes have been characterized (TEM-3 to TEM-26, SHV-2 to SHV-6). These modified β-lactamases are derived by mutation from the well known plasmid-encoded β-lactamases TEM-1 and TEM-2 and also from SHV-1 or other SHV

Table 1: Molecular basis of extended-spectrum beta-lactamases (class A).

Beta-lactamase	Position (amino acid substitution)[a]								Resistance phenotype
	37	102	162	203	235	236	237	261	
TEM-1	Gln	Glu	Arg	Gln	Ala	Gly	Glu	Thr	
TEM-101[b]	Gln		Ser						CAZa
TEM-12 (CAZ-3)	Gln		Ser						CAZa
TEM-10	Gln		Ser				Lys		CAZb
TEM-19 (CTX-2)	Gln					Ser			CTX
TEM-4	Gln	Lys				Ser		Met	CTX
TEM-9 (RHH-1)	Gln	Lys	Ser					Met	CAZb
TEM-5 (CAZ-1)	Gln		Ser		Thr		Lys		CAZb
TEM-6	Gln	Lys	His						CAZb
TEM-2	Lys	Glu	Arg	Gln	Ala	Gly	Glu	Thr	
TEM-14	Lys	Lys						Met	
TEM-3 (CTX-1)	Lys	Lys				Ser			CTX
TEM-7	Lys		Ser						CAZa
TEM-8 (CAZ-2)	Lys	Lys	Ser			Ser			CAZa
TEM-24 (CAZ-6)	Lys	Lys	Ser		Thr		Lys		CAZb
TEM-18	Lys	Lys							CTX
TEM-11	Lys		His						CAZa
TEM-16 (CAZ-7)	Lys	Lys	His						CAZb
SHV-1	Gln	Asp	Arg	Arg	Ala	Gly	Glu	Leu	
SHV-2	Gln					Ser			CTX
SHV-5 (CAZ-4)	Gln					Ser	Lys		CAZb
SHV-3	Gln			Leu		Ser			CTX
SHV-4 (CAZ-5)	Gln			Leu		Ser	Lys		CAZb

[a] Amino acid residues are numbered as described by Sutcliffe for TEM-1, and should be numbered two less for SHV types (review in 1, 2).
[b] In vitro mutant (43).

type β-lactamases of *Klebsiella pneumoniae* (16, 19–24).

Table 1 shows several examples of amino acid substitutions for TEM-1, TEM-2 and SHV type derived enzymes. These changes occurred in positions close to the active site of enzyme, resulting in a better affinity of the modified enzyme for β-lactams, including oxyimino β-lactams (cefotaxime, ceftazidime, aztreonam). Several major features are noteworthy.

Based on the number of substitutions, it is possible to characterize different levels of resistance, which explains some of the original names of the extended-spectrum β-lactamases (e.g. CTX-1 or CTX-2 for cefotaxime; CAZ-1, indicating greater resistance to ceftazidime than to cefotaxime) (20, 25–28). At least four resistance phenotypes (CTX, CAZa, CAZb and ATM) have been characterized, based on the number and position of amino acid substitutions (Table 2).

One single mutation resulted in a significant level of resistance to ceftazidime when located in position 162 (numbered as described by Sutcliffe for TEM-1), for example TEM-12 and TEM-7 (CAZa phenotype) (Table 2). When located in position 236 (serine instead of glycine), the resistance phenotype is named CTX. Beta-lactamases with this phenotype, such as TEM-3, SHV-2 and SHV-3, have a low level of resistance to cefotaxime, ceftazidime, ceftriaxone and aztreonam. A high level of resistance to ceftazidime, cefotaxime and aztreonam simultaneously (CAZb phenotype) is related to a greater number of amino acid substitutions, such as for TEM-10, SHV-4 and SHV-5 (Table 2).

It seems that the introduction of cefotaxime was followed in Europe by the selection of the CTX phenotype, i.e. SHV-2, SHV-3 and TEM-3 (28, 29). Use of ceftazidime followed with the emergence of other types (2, 25). In the absence of ceftazidime, no CAZ-type enzymes were recovered in Tunisia (30). The fact that one single mutation produces a low level of resistance to ceftazidime alone (CAZa) or to ceftazidime, cefotaxime, ceftriaxone and aztreonam simultaneously (CTX phenotype) (MICs usually between 1 and 2 μg/ml) is important because the emergence of the lowest level of resistance would not

Table 2: Resistance phenotypes among *Escherichia coli* derivatives producing extended-spectrum beta-lactamases.

Beta-lactamase	MIC (μg/ml)						Resistance phenotype
	CTX	CAZ	ATM	CAZ / CTX	CAZ / ATM	ATM / CTX	
Derived from TEM-1							
TEM-101 (TEM-12)	0.06	4	0.25	66	16	4	CAZa
TEM-12 (CAZ-3)	0.5	32	2	64	16	4	CAZa
TEM-10	1	64	32	64	2	32	CAZb
TEM-19 (CTX-2)	2	1	0.5	0.5	0.5	0.25	CTX
TEM-4	8	16	2	2	8	0.25	CTX
TEM-9 (RHH-1)	2	128	128	64	1	64	CAZb
TEM-5 (CAZ-1)	8	128	8	16	16	1	CAZa
TEM-6	2	512	32	256	16	16	CAZb
Derived from TEM-2							
TEM-7	0.5	64	2	128	32	4	CAZa
TEM-8 (CAZ-2)	2	128	8	64	16	4	CAZa
TEM-24 (CAZ-6)	8	512	128	64	4	16	CAZb
TEM-14	4	8	4	2	2	1	CTX
TEM-3 (CTX-1)	8	16	8	2	2	1	CTX
TEM-18	2	4	2	2	2	1	CTX
TEM-16 (CAZ-7)	1	128	16	128	16	16	CAZb
TEM-22	4	8	128	2	0.06	32	ATM
Derived from SHV types							
SHV-2	2	2	0.5	1	4	0.25	CTX
SHV-5 (CAZ-4)	4	32	32	8	4	8	CAZb
SHV-3	4	2	1	0.5	0.5	0.25	CTX
SHV-4 (CAZ-5)	4	64	32	16	2	16	CAZb

CTX = cefotaxime; CAZ = ceftazidime; ATM = aztreonam.

be detected (inadequate breakpoint). Isolates producing such enzymes are highly resistant to penicillins (amino-, carboxy- and ureidopenicillins) and cephalosporins (e.g. cephalothin, cefamandole, cefuroxime) (1, 2, 31, 32). In fact, in clinical practice the determination of MICs must be performed either as initially proposed, with a high inoculum in broth medium (27) or, more easily, by using the double synergy test (29, 30, 33–35) applied to strains with a low level of resistance to oxyimino cephalosporins.

Most of the strains initially appeared to be susceptible to oxyimino β-lactams such as cefotaxime, as demonstrated either by the determination of MICs (usually between 1 and 2 μg/ml) or by the distribution of diameters of inhibition zone sizes. These enzymes are highly sensitive to β-lactamase inhibitors such as clavulanic acid (1–2, 17, 18, 25, 27, 29, 33, 36–38). We strongly recommend using the double-disk synergy test to detect isolates producing these enzymes: whatever the resistance phenotype and the type of enzyme, a highly synergistic effect has been demonstrated between a disk containing a combination of 20 μg amoxicillin and 10 μg clavulanic acid and a 30 μg disk of ceftazidime, aztreonam, cefotaxime or ceftriaxone.

In a few cases it is impossible to observe this type of synergy for modified enzymes derived from SHV types (SHV-2, SHV-3, SHV-4) because an adequate level of resistance is obtained only when β-lactamase is overproduced by amplification (M.H. Nicolas et al., 30th ICAAC, Atlanta, 1990, Abstract no. 276). In such cases of negative synergy, cefuroxime was found to be the β-lactam of choice for detection of these enzymes (unpublished results). For a high level of resistance related to high synthesis of β-lactamase by IS insertion (39), the synergy test may be negative.

A probable evolution between extended-spectrum types can be deduced from an observation in a hospital in which originally the SHV-3 type was found and later the SHV-4 (33). A similar observation was reported in a patient during a 24 h interval treatment (TEM-12 and TEM-23 with the same amino acid substitutions as those of TEM-10) (40).

Considering the worldwide distribution and the prevalence of enterobacteria producing TEM-1 β-lactamase, e.g. about 50 % of *Escherichia coli* isolates, the selection of mutants with one amino acid substitution will be easy, as demonstrated in patients treated with ceftazidime (40–42). It was recently suggested that selection is particularly facilitated in patients treated with ceftazidime monotherapy (42). It appears that the selection pressure is less for cefotaxime than for ceftazidime when examined in *Escherichia coli* producing TEM-1 or TEM-2 (23, 43). The actual emergence of some CAZ resistance phenotypes (TEM-12 and TEM-10, which derived from TEM-1) following monotherapy could explain the worldwide distribution of such β-lactamase producing isolates. Furthermore, in the absence of rules of designation, some enzymes not proven to be unique, such as MGH-1, MGH-2, MRH-1, YOU-1 and YOU-2, were observed more recently in several areas, but some others did not receive a denomination (2).

Because of the lower prevalence of TEM-2 among strains of enterobacteria such as *Klebsiella pneumoniae* and *Escherichia coli* instead of *Proteus mirabilis*, the extended-spectrum enzymes derived from TEM-2, such as TEM-3, were initially infrequent and limited in certain areas (2). TEM-22, which derived from TEM-3 by additional mutation (P. Courvalin, personal communication), exists in a single isolate (18). A similar limited distribution has also been observed for SHV-3 and, subsequently SHV-4. This is in contrast to SHV-2 and SHV-5, both of which derive from SHV-1, which is produced mostly by *Klebsiella pneumoniae* isolates (1, 2, 30).

Extended-spectrum β-lactamase producing strains of *Klebsiella pneumoniae* have been reported in many countries from several continents, including Europe, Africa, Australia, Asia and Central,

Table 3: Prevalence of resistance to oxyimino cephalosporins in *Klebsiella pneumoniae*.

Country	Year	No. of hospitals	No. (%) of isolates	Reference
France	1988	20	590 (11)	44
France	1988	12	977 (11.5)	45
France	1991	26[a]	676 (10.2)	–[b]
France	1991	39	229 (38)	–[c]
Senegal	1987–88	2	45 (74.6)	46
Greece	1986–89	1	353 (7.4)	47
Turkey	1992	1	(25)	48
UK	1991	1	70 (14.3)	49
USA	1988	26	353 (7.4)	–[d]
Morocco	1988–90	1	330 (21)	–[e]

[a] Non university hospitals.
[b] F. Goldstein, personal communication.
[c] Multicentre ICU survey, Merck Sharpe and Dohme.
[d] A.A. Medeiros et al., 29th ICAAC, Houston, 1989, Abstract no. 670.
[e] A. Benouda et al., 11th Interdisciplinary Meeting on Anti-Infectious Chemotherapy, Paris, 1991, Abstract no. 334/P20.

North and South America. Table 3 reports some frequencies of extended-spectrum β-lactamase producing strains for this bacterial species (44–49, A.A. Medeiros et al. 29th ICAAC, Houston, 1989, Abstract no. 670; A. Benouda et al. 11th Interdisciplinary Meeting on Anti-Infectious Chemotherapy, Paris, 1991, Abstract no. 334/P20), the highest being observed among isolates obtained from intensive care units, as shown by a recent French multicentre survey (MSD ICU multicentre survey). *Klebsiella pneumoniae* is the most common β-lactamase producing organism (> 80 % of enterobacteria examined), followed by other enterobacteria such as *Escherichia coli* and, to a lesser extent, *Citrobacter freundii* and *Enterobacter cloacae* (28, 30, 34, 44, 45, 48). More recently, *Proteus mirabilis* was implicated in one outbreak (P. Nordmann, personal communication). *Salmonella* isolates appeared more frequently among neonates or infants and particularly in developing countries (26, 30). One *Salmonella typhi* isolate producing SHV-2 was also recovered (J.F. Vieu et al., unpublished results).

If *Klebsiella pneumoniae* is a preferential host, this feature was not related to virulence factors among isolates: 3.7 % produced aerobactin, 7 % a mucoid phenotype and 2 % both factors, unrelated to the type of extended-spectrum β-lactamase produced (50). R-plasmid-encoded adhesive factor was also found in some isolates (51). Investigations with the double-disk synergy test revealed the majority of the extended-spectrum β-lactamases to be SHV types in France (35, 44) while TEM types predominated in the USA (2, A.A. Medeiros et al., 29th ICAAC, Houston, 1989, Abstract no. 670). The β-lactamase distribution varied from country to country (1, 2) and according to the method used, such as oligotyping with only TEM probes (52). In France, the TEM-3, SHV-3 and SHV-4 types were predominant, unlike findings observed in other countries, where the types detected have been predominantly SHV-2 and SHV-5 among isolates of *Klebsiella pneumoniae*. Since its discovery in West Germany (53, 54), SHV-2 has been reported in various countries such as Argentina, Australia, Chile, China, France, Greece, Senegal, Spain, Switzerland, Tunisia, Turkey and the USA (29, 36, 38, 46, 49, 53–57).

These enzymes were originally recovered from patients hospitalized in intensive care units (28, 29, 53). The β-lactamase producing isolates were obtained mostly from urine (about 50 % of isolates) but also from blood (around 15 %), pus and wounds (30). In one instance they spread through

Table 4: Other extended-spectrum clavulanate-sensitive beta-lactamases (classes A and D or unknown).

Beta-lactamase	Country	Year isolated or reported (R)	Species	pI	Plasmid					Sequence	
					Name	Transferred by conjugation	Recipient	Mass	Markers	No.	ßla homologies
FEC-1	Japan	1988 (R)	*E. coli*	8.2	pFCX1	yes	*E. coli* CSH2	74–78 MDa			
FPM-1	Japan	1986	*P. mirabilis*	7.2	pPM-1	yes	*E. coli* CSH2		Sm Tc		
MEN-1	France	1989	*E. coli*	8.4	-	yes	*E. coli* C600	85 kb		263 amino acids	72 % *K. oxytoca*
CTX-M	Germany	1990 (R)	*E. coli*	8.9	pMVP-3	yes	*E. coli* A15	160 kb	Tc Tmp Su		
OXA-11	Turkey	1991	*P. aeruginosa*	6.4	pLMH-52	yes	*P. aeruginosa* PU21	100 MDa	AmGmTm	798 bp	> 99 % PSE-2[a]
PER-1[b]	France	1991	*P. aeruginosa*	5.4	-	no	*P. aeruginosa*	-		924 bp	40 % *B. vulgatus*[c]

[a] Derived from PSE-2 by two amino acid substitutions: position 143 (serine for asparagine) and 157 (aspartate for glycine) (5).
[b] Chromosomal location, no transposable element yet demonstrated (70, 72).
[c] 1.1 kb *SnaB*1 probe did not hybridize with TEM, SHV, PSE, ampC *P. aeruginosa*, L1 and L2 *Xanthomonas maltophilia* enzymes.
Am, amikacin; Gm, gentamicin; Tm, tobramycin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline; Tmp, trimethoprim.

Table 5: In vitro susceptibility of *Escherichia coli* derivates (clavulanate-sensitive beta-lactamases) to antimicrobial agents.

Beta-lactamase	MIC µg/ml									
	Ampicillin	Cephalothin	Cefuroxime	Ceftazidime	Cefotaxime	Ceftizoxime	Aztreonam	Cefoxitin	Moxalactam	Imipenem
FEC-1	>400	>400[a]	>400	12.5	200	1.56	25	1.56	0.39	0.78
FPM-1	400	400	400	3.13	100	0.78	–	0.78[b]	–	–
MEN-1	–	–	–	32	128	–	–	4	1	0.5
CTX-M	128	–	1024	2	16	0.25	8	4	–	0.03
OXA-11	>512	–	–	32	0.25	–	32	2	0.5	0.25
PER-1	>512[c]	128	–	256	4	–	128	8	0.5	<0.03

[a] Cephaloridine.
[b] Cefmetazole.
[c] Amoxicillin.

Table 6: Enzymatic properties of clavulanate-sensitive extended-spectrum beta-lactamases.

	Beta-lactamase				
	FEC-1	FPM-1	MEN-1	OXA-11	PER-1
Molecular mass	48 kDa	26 kDa	28 kDa	27.5 kDa	29 kDa
Substrate profile (Vmax rel)					
Benzylpenicillin	–	–	100	100	100
Ampicillin	17	29	–	72	174[a]
Oxacillin	–	–	–	529	–
Carbenicillin	–	8.2	8.2	3.8	7[b]
Cephalothin	198	240	1300	–	473
Cephaloridine	100	100	–	0.6	356
Cefotaxime	23	20	170	1	1510
Ceftazidime	0.13	0.26	1	0.6	2470
Aztreonam	–	–	6.5	–	1
Cefoperazone	2.6	3.9	–	–	–
Cefoxitin	–	0.01[c]	–	<0.1	<0.5
Imipenem	–	–	–	<0.1	0.5
Inhibition profile					
Clavulanate	0.0093 µM[d]	0.15 µM[d]	0.1 µg/ml[e]	4.5 µM[e]	sensitive
Cloxacillin	–	44 µM	resistant	>100 µM	resistant
Imipenem	0.41 µM	0.63 µM	–	–	sensitive

[a] Amoxicillin.
[b] Carbenicillin or ticarcillin.
[c] Cefoxitin or cefmetazole.
[d] Concentration for 50 % inhibition of nitrocefin (I50s).
[e] Concentration for 50 % inhibition of benzylpenicillin (I50s).

a hospital, causing outbreaks, often in intensive care units such as surgical, neurology or medical wards (28, 29). Several types of outbreaks have been reported involving different epidemiological features, such as the spread of a conjugative plasmid (58) and the spread of a *Klebsiella pneumoniae* strain (serovar K25) harbouring a large conjugative plasmid among units of the same hospital or among different hospitals (37, 59).

More recently, several outbreaks indicated a broader dissemination among neonates, elderly patients and even outpatients (60–66). Imported cases have also been reported in the UK, Egypt (67) and France as well as in several other European and African countries (unpublished results, and V. Jarlier, personal communication). These enzymes are usually encoded by transmissible multiresistant plasmids (55, 58, 68). The genes

conferring resistance to β-lactams are usually co-transferred with other resistance markers such as aminoglycosides, including netilmicin and amikacin.

Other Clavulanate-Sensitive Beta-lactamases (Classes A, D or Unknown)

The above group of extended-spectrum β-lactamases is distributed worldwide, however several other enzymes from clinical isolates other than *Klebsiella pneumoniae*, such as *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, were recently reported in several countries (Table 4) (2, 4, 5, 7, 69, 70). These enzymes are plasmid-mediated, unlike PER-1. Nevertheless, β-lactamases such as PSE-4 could be located on the chromosome of *Pseudomonas aeruginosa* because of its transposable nature.

The most striking feature is that some of these enzymes could be derived from class A β-lactamases other than TEM and SHV types, such as the chromosomally mediated OXY type in *Klebsiella oxytoca* (71) and OXA-11, by two amino acid substitutions from PSE-2 (Table 4) (5). The progenitor of PER-1 could be derived from the chromosomal β-lactamase CFXA of *Bacteroides vulgatus* (72).

As indicated in Tables 5 and 6, these enzymes mediated resistance to broad-spectrum penicillins such as ampicillin, ticarcillin and piperacillin as well as to some extended-spectrum cephalosporins (cefotaxime, ceftazidime, cefuroxime) and aztreonam. Nevertheless, the methoxyimino cephalosporins (cefoxitin, moxalactam, cefmetazole) and the carbapenems were highly stable. Finally, the effects of β-lactamase inhibitors were variable, as expressed by the MICs or inhibition profiles in terms of respective inhibitory concentrations (I50s).

FEC-1 and FPM-1, identified as type I oxyimino cephalosporinases, did not confer resistance to ceftizoxime or ceftazidime. Both were highly sensitive to clavulanate (0.0093 and 0.15 μM, respectively) (7, 11). For MEN-1 and CTX-M, high synergy was obtained between clavulanate (2 μg/ml) and cefotaxime (from 32-fold to 256-fold) (4, 69). For two extended-spectrum types observed in a single *Pseudomonas aeruginosa* isolate, the synergistic effect with clavulanate (respectively 4 and 2 μg/ml) combined with ceftazidime was 32-fold in *Escherichia coli* transconju-

Table 7: Extended-spectrum clavulanate-resistant beta-lactamases (class AmpC).

Beta-lactamase	Country	Year isolated or reported (R)	Species	pI	Plasmid					Sequence	
					Name	Transferred by conjugation	Recipient	Mass	Markers	No.	AmpC homologies
MIR-1	USA	1988	*K. pneumoniae*	8.4	pMG230	no	*E. coli* C600	44 kb	Hg	150 bp	90.0 % *E. cloacae*
BIL-1	Pakistan	1989	*E. coli*	8.8		yes	*E. coli* J53-2 *K. oxytoca* *E. cloacae*	80 MDa	Cm Tc		
CMY-1	South Korea	1989 (R)	*K. pneumoniae*	8.0	pMVP-1	yes	*E. coli* A15	96 MDa	Am Tm Cm Tc Su		
CMY-2	Greece	1990	*K. pneumoniae*	8.1	pMVP-2	yes	*E. coli*	170 kb		3020 bp	93.6 % *C. freundii*
MOX-1	Japan	1991	*K. pneumoniae*	8.9	pRMOX1	yes	*E. coli* CSH2	180 kb	Tc	33 amino	54.4 % *P. aeruginosa*
LAT-1	Greece	1993 (R)	*K. pneumoniae*	9.4	pHP15	no	*E. coli* C600	5.3 MDa		acids	

Am, amikacin; Cm, chloramphenicol; Hg, mercury; Tm, tobramycin; Tc, tetracycline; Su, sulphonamides.

Table 8: In vitro susceptibility of *Escherichia coli* derivatives (class C beta-lactamases) to antimicrobial agents.

Beta-lactamase	MIC (μg/ml)									
	Ampicillin	Ampicillin + clavulanate	Carbenicillin	Cephalothin[a]	Cefotaxime	Ceftazidime	Cefoxitin[b]	Moxalactam	Aztreonam	Imipenem
MIR-1	1000	>256	–	–	64	128	>64	64	128	1
BIL-1	>128	R	128	>128	8	16	–	–	4	–
CMY-1	2048	128	128[c]	>1024	64	4	256	8	16	0.25
CMY-2	–	–	–	–	32	128	256	2	64	0.25
MOX-1	>512	–	–	512	>512	16	>512	>512	16	0.5
LAT-1	>128	64	128	–	128	64	64	–	64	1

[a] Cephalothin or cefazolin or cephaloridine.
[b] Cefoxin or cefotetan.
[c] Piperacillin.
R = resistant.

Table 9: Enzymatic properties of class C beta-lactamases.

	Beta-lactamase			
	MIR-1	BIL-1	MOX-1	LAT-1
Inducibility	–		–	–
Substrate profile (Vmax rel)				
Ampicillin	1	<1[a]	40	1
Carbenicillin	<1	<1	–	<1
Cephalothin	122	1.2	–	130
Cephaloridine	100	100	100	100
Cefoxitin	<1	–	–	<1
Cefotaxime	10	<1	201	<1
Ceftazidime	3	<1	1.5	1
Moxalactam	–	–	2.4	–
Aztreonam	–	–	80	–
Inhibition profile[b]				
Clavulanate	210 nM	362 μM	5.6 μM	800 nM
Cloxacillin[c]	5 nM	8.5 μM	0.35 μM	1 nM
Aztreonam	0.4 nM	–	–	0.2 nM
Cefoxitin	6–10 nM	4.1 μM	–	6.3 nM

[a] Vmax/Km.
[b] Concentration for 50 % inhibition of nitrocefin, except for MOX-1 (cephaloridine, Ki).
[c] Cloxacillin or ampicillin.

gant producing OXA-11 (5) and 2133-fold for PER-1 in an *Escherichia coli* transformant (70).

Cephalosporinases/Cefamycinases (Class C)

A cephalosporinase is usually defined as an enzyme that hydrolyzes cephalosporins (e.g. cephaloridine, cephalothin) four to eight times more effectively than ampicillin (11). Additionally, such enzymes are strongly inhibited by ampicillin, carbenicillin, cloxacillin and aztreonam (inhibition concentration 50 % or I50s < 1 μM) but not by a low concentration of clavulanate (I50s 100-fold higher) (14).

The role of chromosomal enzymes, produced naturally by *Enterobacter* spp., *Citrobacter freundii* and *Serratia marcescens* isolates, is well documented. When overproduced, these enzymes cause the strains to acquire resistance to oxyimino and methoxy β-lactams (cefamycins) (73, 74). Fortunately, only a few AmpC-related β-lactamases mediated by R plasmids have been reported in different countries such as Greece,

Japan, Pakistan, and the USA (Table 7) (2, 3, 6, 8, 9,10, 75, A. Bauernfeind et al., 30th ICAAC, Atlanta, 1990, Abstract no. 190). This minor cluster of plasmid-mediated β-lactamases recently characterized produced resistance to β-lactams, e.g. oxyimino and methoxyimino β-lactams, including cefoxitin, cefotetan, cefmetazole and moxalactam. Their spectrum most closely resembles those of the chromosomal cephalosporinases (Table 8). Otherwise, such enzymes were resistant to clavulanate.

These extended-spectrum clavulanate-resistant β-lactamases, e.g. MIR-1, BIL-1, MOX-1 and LAT-1, showed the characteristic properties of cephalosporinases (Table 9), based on molecular mass (> 35 kDa), pI (alkaline), substrate profile (preferential hydrolysis of cephalosporins), inhibition profile (highly sensitive to cloxacillin and/or aztreonam) and poor inhibition by clavulanate. It could be suggested that such enzymes belong to the group of serine β-lactamases, generally encoded on the chromosome of gram-negative bacteria. However, such β-lactamases encoded by the bacterial chromosome belonging to class C are usually inducible under the regulation of AmpD, AmpR and AmpG in gram-negative bacteria, including *Enterobacter cloacae* and *Citrobacter freundii*. Nevertheless, the production of such novel enzymes was expressed constitutively in *Escherichia coli* (6, 8,10).

Finally, it was suggested that the total amino acid sequence of such enzymes may share some homologies with that of known class C enzymes (Table 7). The β-lactamase MIR-1 showed homology at the amino acid sequence level with *Enterobacter cloacae* AmpC (8). The CMY-2 enzyme was found to show a high degree of DNA homology with the chromosomal AmpC of *Citrobacter freundii* (A. Bauernfeind et al., 32nd ICAAC, Anaheim, 1992, Abstract no. 1268). MOX-1 showed significant homology in its N terminal amino acid sequence with AmpC of *Pseudomonas aeruginosa* (6). MOX-1 showed a closer relationship to the chromosomal AmpC of *Pseudomonas aeruginosa* PAO1 than to those of enteric bacteria, but the bla_{MOX-1} probe did not hybridize with the chromosomal ampC gene of *Pseudomonas aeruginosa* PAO1 (6).

Other plasmid-encoded β-lactamases (BIL-1, LAT-1) were considered to be derivatives of AmpC-type β-lactamase other than that of *Enterobacter cloacae*, but no amino acid or nucleotide sequences were reported. Furthermore, these enzymes do not belong to a TEM- or SHV-related type. This new aspect of resistance, reported among a few clinical *Klebsiella pneumoniae* isolates and one *Escherichia coli* isolate, is not a major dilemma in hospital-acquired infections with gram-negative bacteria. Only one outbreak of MIR-1 was reported (8). However, it remains unknown why only a few plasmid-mediated AmpC-type β-lactamases have been found. Such AmpC enzymes must be clearly differentiated from other clavulanate-sensitive extended-spectrum β-lactamases to provide another therapeutic choice, and such enzymes were obviously undetectable by the double-disk synergy test (76).

Metalloenzymes (Carbapenemases)

Beta-lactamase-mediated resistance to carbapenems is still very rare in clinically important species but may pose a threat in the future (77). The chromosomally mediated metalloenzymes, found among isolates of *Xanthomonas maltophilia* (L1), *Aeromonas sobria* and *Bacillus cereus*, have the broadest substrate profile among β-lactamases. The profile includes penicillins, oxyimino cephalosporins, methoxyimino cephalosporins and carbapenems. Furthermore, these enzymes were resistant to β-lactamase inhibitors such as clavulanate, sulbactam and tazobactam but inac-

Table 10: Extended-spectrum clavulanate-resistant metallo beta-lactamases (MET).

Beta-lactamase	Country	Year isolated or reported (R)	Species	pI	Plasmid				
					Name	Transferred by conjugation	Recipient	Mass	Markers
MET A[a]	Japan	1988	*P. aeruginosa*	9.0	pMS350	yes	*P. aeruginosa*	31 MDa	Gm Su
MET B	Japan	1992 (R)	*B. fragilis*		pBFUK1	yes	*B. fragilis* TM4000	13.6 kb	

[a] MET for metalloenzyme (14).
Gm, gentamicin; Su, sulphonamides.

tivated by chelating agents such as EDTA because of an active-site zinc ion (77).

Thus far, only two isolates (1 *Pseudomonas aeruginosa*, 1 *Bacteroides fragilis*), both recovered in Japan and obtained by conjugation or a conjugation system, have been found to produce this type of extended-spectrum β-lactamase (type II oxyimino cephalosporinases or CXases) (Table 10) (12, 13). As shown in Tables 11 and 12, such plasmid-encoded enzymes mediate a broad spectrum of resistance to β-lactams, including oxyimino cephalosporins, methoxyimino cephalosporins and carbapenems.

Conclusions

The development of highly stable extended-spectrum cephalosporins at the beginning of the 1980s was a major therapeutic advance. Within a few years, however, at least 30 types of transferable extended-spectrum β-lactamases had been identified, mainly in nosocomial isolates of *Klebsiella pneumoniae*. These enzymes have been shown to be derived from SHV or TEM type β-lactamases by one or more amino acid substitutions. Based on the level of resistance to cefotaxime, cefta-

Table 11: In vitro susceptibility of metalloenzymes (MET) to antimicrobial agents.

Beta-lactamase	MIC (μg/ml)												
	Ampicillin	Ampicillin + clavulanate	Carbenicillin	Piperacillin	Cefoperazone	Cefoperazone + sulbactam	Cefotaxime	Ceftazidime	Cefoxitin	Cefotetan	Moxalactam	Aztreonam	Imipenem	Meropenem
MET A[a]	–	–	> 400	3.13	200	–	–	400	–	–	> 400	3.13	12.5	100
MET B[b]	200	50	–	50	> 200	400	100	–	25	100	100	–	100	–

[a] *Pseudomonas aeruginosa*, recipient strain PAO1.
[b] *Bacteroides fragilis*, recipient strain 1073.

Table 12: Enzymatic properties of metallo beta-lactamases (MET).

	MET A	MET B
Molecular weight	28 kDa	–
Substrate profile (Vmax rel)		
Ampicillin	215	104
Carbenicillin	391	–
Piperacillin	145	
Cephalothin	113	–
Cephaloridine	100	100
Cefotaxime	22	84
Ceftazidime	20	–
Cefoxitin	51	7
Moxalactam	193	146
Aztreonam	< 1	ND
Imipenem	166	120
Meropenem	37	146
Inhibition profile		
Clavulanate	0[a]	–[b]
EDTA	100[a]	+++[b]
Activation Zn2+ (1 mM)	54 %	

[a] Percent inhibition tested at 100 μM.
[b] –, no inhibition at 500 μM; +++ excellent inhibition at 100 μM.
ND, not detected.

zidime and aztreonam, at least four susceptibility patterns have been characterized (CTX, CAZa, CAZb and ATM) in relation to the type and location(s) of amino acid substitution. An evolution of resistance (e.g. TEM-10 to TEM-12, SHV-2 to SHV-5, SHV-3 to SHV-4) has been related to the number of amino acid substitutions.

Clavulanate-sensitive enzymes, with an expanded spectrum of resistance, are distributed worldwide and their prevalence is highly variable. Within this group of clavulanate-sensitive enzymes, the most novel feature is that some of these enzymes could be derived from class A β-lactamases other than TEM and SHV types, such as MEN-1 (from *Klebsiella oxytoca* chromosomal enzyme) and OXA-11 in *Pseudomonas aeruginosa* (from PSE-2 by two amino acid substitutions).

In some countries a novel group of plasmid-encoded extended-spectrum β-lactamases, including clavulanate-resistant cephalosporinases or cefamycinases or class C β-lactamases (usually chromosomally mediated), have been identified from single clinical isolates since 1988. These enzymes (MIR-1, BIL-1, CMY-1, CMY-2, MOX-1, LAT-1) have an extended spectrum of inactivation which includes methoxyimino cephalosporins but not carbapenems. Carbapenemases are to be the next generation of β-lactamases (77), but to date, only two plasmid-encoded metalloenzymes have been reported.

References

1. **Philippon A, Labia R, Jacoby GA:** Extended-spectrum β-lactamases. Antimicrobial Agents and Chemotherapy 1989, 33: 1131–1136.
2. **Jacoby GA, Medeiros AA:** More extended-spectrum β-lactamases. Antimicrobial Agents and Chemotherapy 1991, 35: 1697–1704.
3. **Bauernfeind A, Chong Y, Schweighart S:** Extended-broad-spectrum β-lactamase in *Klebsiella pneumoniae* including resistance to cephamycins. Infection 1989, 17: 316–321.
4. **Bernard H, Tancrède C, Livrelli V, Morand A, Barthélémy M, Labia R:** A novel plasmid-mediated extended-spectrum β-lactamase not derived from TEM- or SHV- type enzymes. Journal of Antimicrobial Chemotherapy 1992, 28: 590–592.
5. **Hall LMC, Livermore DM, Gür D, Akova M, Akalin HE:** OXA-11, an extended-spectrum variant of OXA-10 (PSE-2) β-lactamase from *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy 1993, 37: 1637–1644.
6. **Horii T, Arakawa Y, Ohta M, Ichiyama S, Wacharotayankun R, Kato N:** Plasmid-mediated AmpC-type β-lactamase isolated from *Klebsiella pneumoniae* confers resistance to broad-spectrum β-lactams, including moxalactam. Antimicrobial Agents and Chemotherapy 1993, 37: 984–990.
7. **Matsumoto Y, Ikeda F, Kamimura T, Yokota Y, Mine Y:** Novel plasmid-mediated β-lactamase from *Escherichia coli* that inactivates oxyimino-cephalosporins. Antimicrobial Agents and Chemotherapy 1988, 32: 1243–1246.
8. **Papanicolaou GA, Medeiros AA, Jacoby GA:** Novel plasmid-mediated β-lactamase (MIR-1) conferring resistance to oxyimino- and a-methoxy β-lactams in clinical isolates of *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy 1990, 34: 2200–2209.
9. **Payne DJ, Woodford N, Amyes SGB:** Characterization of the plasmid-mediated β-lactamase BIL-1. Journal of Antimicrobial Chemotherapy 1992, 30: 119–127.
10. **Tzouvelekis LS, Tzelepi E, Mentis AF, Tsakris A:** Identification of a novel plasmid-mediated β-lactamase with chromosomal cephalosporinase characteristics from *Klebsiella pneumoniae*. Journal of Antimicrobial Chemotherapy 1993, 31: 645–654.
11. **Watanabe Y, Yokota T, Higashi Y, Wakai Y, Mine Y:** In vitro and in vivo transferable β-lactam resistance due to a new plasmid-mediated oxyimino cephalosporinase from a clinical isolate of *Proteus mirabilis*. Microbiology and Immunology 1991, 35: 87–97.
12. **Bandoh K, Watanabe K, Muto Y, Tanaka Y, Kato N, Ueno K:** Conjugal transfer of imipenem resistance in *Bacteroides fragilis*. Journal of Antibiotics 1992, 45: 542–547.
13. **Watanabe Y, Iyobe S, Inoue M, Mitsuhashi S:** Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy 1991, 35: 147–151.
14. **Bush K:** A Classification of β-lactamases: groups 1, 2a, 2b, and 2b'. Antimicrobial Agents and Chemotherapy 1989, 33: 264–270.
15. **Ambler RP:** The structure of β-lactamases. Philosophical Transactions of the Royal Society of London (B Biological Sciences) 1980, 289: 321–331.
16. **Huletsky A, Couture F, Levesque RC:** Nucleotide sequence and phylogeny of SHV-2 β-lactamase. Antimicrobial Agents and Chemotherapy 1990, 34: 1725–1732.
17. **Arlet G, Rouveau M, Bengoufa D, Nicolas MH, Philippon A:** Novel transferable extended-spectrum β-lactamase (SHV-6) from *Klebsiella pneumoniae* conferring selective resistance to ceftazidime. FEMS Microbiological Letters 1991, 81: 57–62.
18. **Arlet G, Rouveau M, Fournier G, Lagrange PH, Philippon A:** Novel, plasmid-encoded, TEM-derived extended-spectrum β-lactamase in *Klebsiella pneumoniae* conferring higher resistance to aztreonam than to extended-spectrum cephalosporins. Antimicrobial Agents and Chemotherapy 1993, 37: 2020–2023.
19. **Barthélémy M, Peduzzi J, Ben Yaghlane H, Labia R:** Single amino acid substitution between SHV-1 β-lactamase and cefotaxime-hydrolyzing SHV-2 enzyme. FEBS Microbiological Letters 1988, 231: 217–220.
20. **Chanal C, Poupart MC, Sirot D, Labia R, Sirot J, Cluzel RA:** Nucleotide sequences of CAZ-2, CAZ-6, and CAZ-7 β-lactamase genes. Antimicrobial Agents and Chemotherapy 1992, 36: 1817–1820.
21. **Collatz E, Tran Van Nhieu G, Billot-Klein D, Williamson R, Gutmann L:** Substitution of serine for arginine in position 162 of TEM-type β-lactamases extends the substrate profile of mutant enzymes, TEM-7 and TEM-101, to ceftazidime and aztreonam. Gene 1989, 78: 349–354.
22. **Nicolas MH, Jarlier V, Philippon A, Cole S:** Molecular cloning of the gene SHV-3 responsible for transferable cefotaxime resistance in clinical isolates of *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy 1989, 33: 2096–2100.

23. **Sougakoff W, Goussard S, Gerbaud G, Courvalin P:** Plasmid-mediated-resistance to third-generation cephalosporins due to point mutations in TEM-type penicillinase genes. Reviews of Infectious Diseases 1988, 10: 879–884.
24. **Sougakoff W, Petit A, Goussard J, Sirot D, Buré A, Courvalin P:** Characterization of the plasmid genes blaT-4 and blaT-5, which encode the broad spectrum β-lactamases TEM-4 and TEM-5 in *Enterobacteriaceae*. Gene 1989, 78: 339–348.
25. **Chanal CM, Sirot DL, Petit A, Labia R, Morand A, Sirot JL, Cluzel RA:** Multiplicity of TEM-derived β-lactamases from *Klebsiella pneumoniae* isolated at the same hospital and relationships between the responsible plasmids. Antimicrobial Agents and Chemotherapy 1989, 33: 1915–1920.
26. **Poupart MC, Chanal C, Sirot D, Labia R, Sirot J:** Identification of CTX-2, a novel cefotaximase from a *Salmonella mbandaka* isolate. Antimicrobial Agents and Chemotherapy 1991, 35: 1498–1500.
27. **Sirot D, Sirot J, Labia R, Morand A, Courvalin P, Darfeuille-Michaud A, Perroux R, Cluzel R:** Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*. Identification of CTX-1, a novel beta-lactamase. Journal of Antimicrobial Chemotherapy 1987, 20: 323–334.
28. **Sirot J, Chanal C, Petit A, Sirot D, Labia R, Gerbaud G:** *Klebsiella pneumoniae* and other *Enterobacteriaceae* producing novel plasmid-mediated beta-lactamases markedly active against third-generation cephalosporins: epidemiologic studies. Reviews of Infectious Diseases 1988, 10: 850–859.
29. **Brun-Buisson C, Legrand P, Philippon A, Montravers F, Ansquer M, Duval J:** Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. Lancet 1987, ii: 302–306.
30. **Philippon A, Ben Redjeb S, Fournier G, Ben Hassen A:** Epidemiology of extended-spectrum β-lactamases. Infection 1989, 17: 347–354.
31. **Jacoby GA, Carreras I:** Activities of β-lactam antibiotics against *Escherichia coli* strains producing extended-spectrum β-lactamases. Antimicrobial Agents and Chemotherapy 1990, 34: 858–862.
32. **Kitzis MD, Liassine N, Ferré B, Gutmann L, Acar JF, Goldstein F:** In vitro activities of 15 oral β-lactams against *Klebsiella pneumoniae* harboring new extended-spectrum β-lactamases. Antimicrobial Agents and Chemotherapy 1990, 34: 1783–1786.
33. **Jarlier V, Nicolas MH, Fournier G, Philippon A:** Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactams in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Reviews of Infectious Diseases 1988, 10: 867–878.
34. **Legrand P, Fournier G, Buré A, Jarlier V, Nicolas MH, Decré D, Duval J, Philippon A:** Detection and distribution of extended broad-spectrum β-lactamases in *Enterobacteriacae* in four French hospitals. European Journal of Clinical Microbiology and Infectious Diseases 1989, 8: 527–529.
35. **Philippon A, Fournier G, Paul G, Vedel G, Névot P:** Détection et distribution des β-lactamases à spectre élargi chez les entérobactéries. Médecine et Maladies Infectieuses 1988, 12: 869–876.
36. **Ben Redjeb S, Ben Yaghlane H, Boujnah A, Philippon A, Labia R:** Synergy between clavulanic acid and newer β-lactams on 9 clinical isolates of *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhimurium* resistant to third generation cephalosporins. Journal of Antimicrobial Chemotherapy 1988, 21: 263–266.
37. **Buré A, Legrand P, Arlet G, Jarlier V, Paul G, Philippon A:** Dissemination of *Klebsiella pneumoniae* serotype K25 harbouring a new transferable enzymatic resistance to third generation cephalosporins and aztreonam in five French hospitals. European Journal of Clinical Microbiology and Infectious Diseases 1988, 7: 780–782.
38. **Gutmann L, Ferré B, Goldstein F, Risk N, Acar JF, Collatz E:** SHV-5, A novel SHV-type β-lactamase that hydrolyzes broad-spectrum cephalosporins and monobactams. Antimicrobial Agents and Chemotherapy 1989, 33: 951–956.
39. **Goussard S, Sougakoff W, Mabilat C, Bauernfeind A, Courvalin P:** An IS1-like element is responsible for high-level synthesis of extended-spectrum β-lactamase TEM-6 in *Enterobacteriaceae*. Journal of General Microbiology 1991, 137: 2681–2687.
40. **Vedel G, Mabilat C, Goussard S, Picard B, Fournier G, Gilly L, Paul G, Philippon A:** Two variants of transferable extended-spectrum TEM-β-lactamase successively isolated from an *Escherichia coli* isolate. FEMS Microbiological Letters 1992, 93: 161–166.
41. **Smith CE, Tillman BS, Howell AW, Longfield RN, Jorgensen JH:** Failure of ceftazidime-amikacin therapy for bacteremia and meningitis due to *Klebsiella pneumoniae* producing an extended-spectrum β-lactamase. Antimicrobial Agents and Chemotherapy 1990, 34: 1290–1293.
42. **Naumoski L, Ouinn JP, Miyashiro D, Patel M, Bush K, Singer SB, Graves D, Palzkill T, Arvin AN:** Outbreak of ceftazidime resistance due to a novel extended-spectrum β-lactamase in isolates from cancer patients. Antimicrobial Agents and Chemotherapy 1992, 36: 1991–1996.
43. **Gutmann L, Kitzis MD, Billot-Klein D, Goldstein F, Tran Van Nhieu G, Lu T, Carlet J, Collatz E, Williamsom R:** Plasmid-mediated β-lactamase (TEM-7) involved in resistance to ceftazidime and aztreonam. Reviews of Infectious Diseases 1988, 10: 860–866.
44. **Thabaut A, Acar J, Allouch G, Arlet G, Berardi-Grassias L, Bergogne-Bérézin E, Brun Y, Buisson Y, Chabanon G, Cluzel R, Courtieu A, Dabernat H, Duval J, Fleurette J, Ghnassia JC, Jarlier V, Meyran M, Monteil H, Petithory JC, Philippon A, Reverdy ME, Reynaud A, Sedaillan A, Sirot J, Werneburg B:** Fréquence et distribution des béta-lactamases chez 1792 souches de *Klebsiella pneumoniae* isolées en France entre 1985 et 1988. Pathologie et Biologie 1990, 38: 459–463.
45. **Sirot DL, Goldstein FW, Soussy CJ, Courtieu AL, Husson MO, Lemozy J, Meyran M, Morel C, Perez R, Quentin-Noury C, Reverdy ME, Scheftel JM, Rosembaum M, Rezvany Y:** Resistance to cefotaxime and seven other β-lactams in members of the family *Enterobacteriaceae*: a 3-year survey in France. Antimicrobial Agents and Chemotherapy 1993, 36: 1677–1681.
46. **Richard C, Philippon A, M'Boup S, Vieu JF:** Epidémiologie des infections pédiatriques à *Klebsiella* dans deux hôpitaux de Dakar: production de β-lactamases à spectre élargi (1987–1988). Médecine et Maladies Infectieuses 1989, 19: 753–759.
47. **Vatopoulos AC, Philippon A, Tzouvelekis LS, Legakis NJ, Komninou Z:** Prevalence of a transferable SHV-5 beta-lactamase in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Greece. Journal of Antimicrobial Chemotherapy 1991, 26: 635–648.

48. **Gür D, Pitt TL, Hall LM, Erdal Akalin H, Livermore DM:** Diversity of klebsiellae with extended-spectrum β-lactamases at a Turkish university hospital. Journal of Hospital Infection 1992, 22: 163–178.
49. **Liu PYF, Gür D, Hall LMC, Livermore D:** Survey of the prevalence of β-lactamases amongst 1000 gram-negative bacilli isolated consecutively at the Royal London Hospital. Journal of Antimicrobial Chemotherapy 1992, 30: 429–447.
50. **Vernet V, Madoulet C, Bajolet O, Philippon A:** Incidence of two virulence factors (aerobactin and mucoid phenotype) among 190 clinical isolates of *Klebsiella pneumoniae* producing extended-spectrum β-lactamases. FEMS Microbiological Letters 1992, 96: 1–6.
51. **Darfeuille-Michaud A, Jallat C, Aubel D, Sirot D, Rich C, Sirot J, Joly B:** R-plasmid-encoded adhesive factor in *Klebsiella pneumoniae* strains responsible for human nosocomial infections. Infection and Immunity 1992, 60: 44–55.
52. **Mabilat C, Courvalin P:** Development of "oligotyping" for characterization and molecular epidemiology of TEM β-lactamases in members of the family *Enterobacteriaceae*. Antimicrobial Agents and Chemotherapy 1990, 34: 2210–2216.
53. **Shah PM, Stille W:** *Escherichia coli* and *Klebsiella pneumoniae* strains more susceptible to cefoxitin than to third generation cephalosporins. Journal of Antimicrobial Chemotherapy 1983, 11: 597–598.
54. **Kliebe C, Nies BA, Meyer JF, Tolxdorff-Neutzling RM, Wiedemann B:** Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrobial Agents and Chemotherapy 1985, 28: 302–307.
55. **Fernandez-Rodriguez A, Canton R, Perez-Diaz JC, Martinez Beltran J, Picazo JJ, Baquero F:** Aminoglycoside-modifying enzymes in clinical isolates harboring extended-spectrum β-lactamases. Antimicrobial Agents and Chemotherapy 1992, 36: 2536–2538.
56. **Labia R, Morand A, Tiwari K, Pitton JS, Sirot D, Sirot J:** Kinetic properties of two plasmid-mediated β-lactamases from *Klebsiella pneumoniae* with a strong activity against third-generation cephalosporins. Journal of Antimicrobial Chemotherapy 1988, 21: 301–307.
57. **Mulgrave L:** Extended broad-spectrum β-lactamases in Australia. Medical Journal of Australia 1990, 152: 444–445.
58. **Petit AG, Gerbaud G, Sirot D, Courvalin P, Sirot J:** Molecular epidemiology of TEM-3 (CTX-1) β-lactamase. Antimicrobial Agents and Chemotherapy 1990, 34: 219–224.
59. **Arlet G, Sanson-le Pors MJ, Rouveau M, Fournier G, Marie O, Schlemmer B, Philippon A:** Nosocomial outbreak of infections due to *Klebsiella pneumoniae* that produce SHV-4 β-lactamase. European Journal of Clinical Microbiology and Infectious Diseases 1990, 9: 797–803.
60. **Bingen E, Desjardins P, Arlet G, Bourgeois F, Mariani-Kurkdjian P, Lambert-Zechovsky N, Denamur E, Philippon, Elion J:** Molecular epidemiology of plasmid spread among extended broad-spectrum β-lactamase producing *Klebsiella pneumoniae* in a pediatric hospital. Journal of Clinical Microbiology 1993, 31: 179–184.
61. **Coovadia YM, Johnson AP, Bhana RH, Hutchinson GR, George RC, Hafferjee IE:** Multiresistant *Klebsiella pneumoniae* in a neonatal nursery: the importance of maintenance of infection control policies and procedures in the prevention of outbreaks. Journal of Hospital Infection 1992, 22: 197–205.
62. **de Champs C, Sirot D, Chanal C, Poupart MC, Dumas MP, Sirot J:** Concomitant dissemination of three extended-spectrum β-lactamases among different *Enterobacteriaceae* isolated in a French hospital. Journal of Antimicrobial Chemotherapy 1991, 27: 441–457.
63. **Hammani A, Arlet G, Ben Redjeb S, Fournier G, Ben Hassen A, Rekik A, Philippon A:** Nosocomial outbreak of acute gastroenteritis caused by multiply drug-resistant *Salmonella wien* producing SHV-2 β-lactamase in a neonatal intensive care unit. European Journal of Clinical Microbiology and Infectious Diseases 1991, 10: 641–646.
64. **Meyer KS, Urban C, Eagan JA, Berger BJ, Rahal JJ:** Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. Annals of Internal Medicine 1993, 119: 353–358.
65. **Rasmussen BA, Bradford PA, Quinn JP, Wiener J, Weinstein RA, Bush K:** Genetically diverse ceftazidime-resistant isolates from a single center: biochemical and genetic characterization of TEM-10 β-lactamase encoded by different nucleotide sequences. Antimicrobial Agents and Chemotherapy 1993, 37: 1989–1992.
66. **Rice LB, Willey SH, Papanicoualou GA, Medeiros AA, Eliopoulos GM, Moellering RC, Jacoby GA:** Outbreak of ceftazidime resistance caused by extended-spectrum β-lactamases at a Massachusetts chronic-care facility. Antimicrobial Agents and Chemotherapy 1990, 34: 2193–2199.
67. **Shannon KP, King A, Phillips I, Nicolas MH, Philippon A:** Import of organisms producing broad-spectrum SHV-group beta-lactamases into the United Kingdom. Journal of Antimicrobial Chemotherapy 1990, 25: 343–351.
68. **Jacoby GA, Sutton L:** Properties of plasmids responsible for production of extended-spectrum β-lactamases. Antimicrobial Agents and Chemotherapy 1991, 35: 164–169.
69. **Bauernfeind A, Grimm H, Schweighart S:** A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli*, Infection 1990, 18: 294–298.
70. **Nordmann P, Ronco E, Naas T, Duport C, Michel-Briand Y, Labia R:** Characterization of a novel extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy 1993, 37: 962–969.
71. **Barthélémy M, Péduzzi J, Bernard H, Tancrède C, Labia R:** Close amino acid sequence relationship between the new plasmid-mediated extended-spectrum β-lactamase MEN-1 and chromosomally encoded enzymes of *Klebsiella oxytoca*. Biochimica et Biophysica Acta 1992, 1122: 15–22.
72. **Nordmann P, Naas T:** Sequence analysis of PER-1 extended-spectrum β-lactamase from *Pseudomonas aeruginosa* and comparison with class A β-lactamases. Antimicrobial Agents and Chemotherapy, 1994, 38: 104–114.
73. **Sanders CC:** Chromosomal cephalosporinases responsible for multiple resistance to newer β-lactam antibiotics. Annual Review of Microbiology 1987, 41: 573–593.
74. **Sanders CC, Sanders WE:** β-lactam resistance in gram-negative bacteria: global trends and clinical impact. Clinical Infectious Diseases 1992, 15: 824–839.
75. **Woodford N, Payne DJ, Johnson AP, Weinbren MJ, Perinpanayagam RM, George RC, Cookson BD, Amyes SGB:** Transferable cephalosporin resistance not inhibited by clavulanate in *Escherichia coli*. Lancet 1990, 336: 253.
76. **Thomson KS, Sanders CC:** Detection of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae*: comparison of the double-disk and three-dimensional tests. Antimicrobial Agents and Chemotherapy 1992, 36: 1877–1882.
77. **Livermore D:** Carbapenemases: the next generation of β-lactamases? ASM News 1993, 59: 129–135.