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MINIREVIEW

Plasmid-Determined AmpC-Type β-Lactamases

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The predominant mechanism for resistance to β-lactam antibiotics in gram-negative bacteria is the synthesis of β-lactamase. To meet this challenge, β-lactams with greater β-lactamase stability, including cephalosporins, carbapenems, and monobactams, were introduced in the 1980s. Resistance appeared initially in organisms such as *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, and *Pseudomonas aeruginosa* that could, by mutation, overproduce their chromosomal AmpC (also termed class C or group 1) β-lactamase, thus providing resistance to both oxyimino- and 7-α-methoxy-cephalosporins and monobactams (74).

Later, resistance appeared in bacterial species that lack an inducible AmpC enzyme, such as *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* spp., and *Proteus mirabilis*, and this resistance was found to be mediated by plasmids encoding extended-spectrum β-lactamases (ESBLs), which are enzymes that arose by mutations in TEM or SHV β-lactamases of more limited hydrolytic capacity (39, 40, 68). Such resistance included oxyimino-cephalosporins and monobactams but not 7-α-methoxy-cephalosporins and was blocked by clavulanate, sulbactam, or tazobactam, which are inhibitors that are generally ineffective against class C enzymes (49, 75).

With continuing use of 7-α-methoxy-cephalosporins (cefoxitin and cefotetan) and the clinical introduction of β-lactamase inhibitor combinations (clavulanate with amoxicillin or ticarcillin, sulbactam with ampicillin, and tazobactam with piperacillin), plasmids encoding class C β-lactamases appeared (56). Like their counterpart on the chromosome, such enzymes provided a broader spectrum of resistance than ESBLs and were not blocked by commercially available inhibitors.

Furthermore, in a strain with decreased outer membrane permeability such enzymes can provide resistance to carbapenems as well, as has been observed with clinical isolates of *K. pneumoniae* during an outbreak in New York (13) and in individual isolates of *E. coli* in the United Kingdom (78) and of *K. pneumoniae* in Sweden (17).

HISTORY

AmpC β-lactamases, demonstrated or presumed to be chromosomally mediated, have been described in *Acinetobacter* spp., *Aeromonas* spp., *Chromobacterium violaceum*, *C. freundii*, *Enterobacter* spp., *E. coli*, *Hafnia alvei*, *Lysobacter lactamgenus*, *Morganella morganii*, *Ochrobactrum anthropi*, *Proteus rettgeri*, *Providencia stuartii*, *P. aeruginosa*, *Psychrobacter immobilis*, *Rhodobacter sphaeroides*, *S. marcescens*, and *Yersinia enterocolitica*. In many genera, AmpC is inducible via a system involving *ampD, ampG, ampR,* and intermediates in peptidoglycan recycling (38, 86). The *ampC* gene of *E. coli* is normally expressed at a low level, regulated by a growth rate-dependent attenuation mechanism (42) but not by induction, since *ampR* is missing (35). In *Shigella flexneri* and *Shigella dysenteriae*, *ampC* is included in a large deletion (55). An *ampC* locus appears on the genetic map of *Salmonella* (76), but the evidence for its existence was indirect and its presence has not been confirmed in the sequenced genomes of *Salmonella enterica* serotypes Typhimurium or Paratyphi (57), so that *Salmonella* is considered to be AmpC$^-$ (56). A chromosomal *ampC* gene is also lacking in *Klebsiella* spp. (57) and *P. mirabilis*.

In 1976, Bobrowski et al. described a plasmid-mediated β-lactamase indistinguishable from the AmpC enzyme of *E. coli* in a strain of *P. mirabilis* (11). Unfortunately, the original plasmid was lost, there was some doubt about the transfer experiments, and molecular studies were not done. In 1982, Levesque et al. reported a plasmid-mediated cephalosporinase in *Achromobacter* spp. (48). Regrettably, the original strain was lost, the β-lactamase gene was not sequenced, and in retrospect the biochemical properties of the enzyme resembled those of a group 2b broad-spectrum enzyme rather than of a group 1 cephalosporinase. In 1983, Knothe et al. reported the transfer of cefoxitin resistance from *S. marcescens* to *Proteus* or *Salmonella* spp., but resistance segregated on transfer to *E. coli* and no biochemical or molecular studies were done (44).

In 1989, Bauernfeind et al. described a *K. pneumoniae* isolate from South Korea that could transfer resistance to cefoxitin and cefotetan as well as to penicillins, oxyimino-cephalosporins, and monobactams to *E. coli* (6). The enzyme, termed CMY-1 for its cephamycinase activity, had an isoelectric point (pI) of 8.0 and was more sensitive to inhibition by sulbactam than by clavulanate or tazobactam, suggesting that it might be a class C enzyme. However, the first proof that a class C β-lactamase had been captured on a plasmid was provided by Papanicolaou et al., who described transmissible resistance to α-methoxy- and oxyimino-β-lactams mediated by an enzyme (MIR-1) with the biochemical properties of a class 1 β-lactamase and showed that part of the MIR-1 gene was 90% identical to the *ampC* gene of *E. cloacae* (65).

Subsequently, plasmid-mediated class C β-lactamases have been discovered worldwide (Table 1). They have been named

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TABLE 1. Chronology of discovery of AmpC plasmid-encoded β-lactamases[a]

Name	Country[b]	Year[c]	Species	pI	Reference(s)
MIR-1	United States	1988	*K. pneumoniae*	8.4	41, 65
CMY-1	South Korea	1988	*K. pneumoniae*	8.0	6, 9
BIL-1	United Kingdom (Pakistan)	1989	*E. coli*	8.8	66, 88
FOX-1	Argentina	1989	*K. pneumoniae*	6.8–7.2	31
CMY-2	Greece	1990	*K. pneumoniae*	9.0	8
	France (Algeria)	1994	*Salmonella senftenberg*	9.0	46
MOX-1	Japan	1991	*K. pneumoniae*	8.9	36
DHA-1	Saudi Arabia	1992	*S. enteritidis*	7.8	26
	France	1998	*K. pneumoniae*	7.8	Verdet et al.[d]
DHA-2	France	1992	*K. pneumoniae*	7.8	24
FOX-2	Germany (Guatemala)	1993	*K. pneumoniae*	6.7	10
LAT-1	Greece	1993	*K. pneumoniae*	9.4	82, 83
FOX-3	Italy	1994	*K. oxytoca*, *K. pneumoniae*	7.25	51
LAT-2	Greece	1994	*K. pneumoniae*, *E. coli*, *E. aerogenes*	9.1	28
ACT-1	United States	1994	*K. pneumoniae*, *E. coli*	9.0	13
MOX-2	France (Greece)	1995	*K. pneumoniae*	9.2	Boyer-Mariotte et al.[e]
CMY-4	Tunisia	1996	*P. mirabilis*	9.2	85
	United Kingdom	1999 (P)	*E. coli*	>8.5	78
	Sweden (India)	1998	*K. pneumoniae*	9.0	17
ACC-1	Germany	1997	*K. pneumoniae*	7.7	7
	France (Tunisia)	1998	*K. pneumoniae*	7.8	60
	Tunisia	1997–2000	*K. pneumoniae*, *P. mirabilis*, *Salmonella* spp.	7.8	71
	France (Tunisia)	2000	*P. mirabilis*, *E. coli*	7.7	30
CMY-3[f]	France	1998 (P)	*P. mirabilis*	9.0	14
LAT-3	Greece	1998 (P)	*E. coli*	8.9	29
LAT-4	Greece	1998 (P)	*E. coli*	9.4	29
CMY-8	Taiwan	1998	*K. pneumoniae*	8.25	91
CMY-5	Sweden	1999 (P)	*K. oxytoca*	8.4	89, 90
FOX-4	Spain	2000 (P)	*E. coli*	6.4	12

[a] Other plasmid-mediated AmpC-type β-lactamases have been described in GenBank but are not yet published, including CMY-6, CMY-7, CMY-9, CMY-10, CMY-11, and FOX-5.
[b] Country of isolation (if different, probable country of origin is indicated in parentheses).
[c] Year of isolation or date of publication (P).
[d] C. Verdet, N. Boutros, B. Salauze, A. Rossier, T. Lambert, A. Philippon, and G. Arlet. Abstr. 20th Réunion Interdisciplinaire Chimiothérapie Anti-Infectieuse, abstr. 24/C4, 2000.
[e] S. Boyer-Mariotte, L. Raskine, B. Hanau, A. Philippon, M.M. Sanson-LePors, and G. Arlet, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-7, 1998.
[f] Chromosomal location.

with inconsistency typical of β-lactamase nomenclature according to the resistance produced to cephamycins (CMY), cefoxitin (FOX), and moxalactam (MOX) or latamoxef (LAT), to the type of β-lactamase, such as AmpC type (ACT) or Ambler class C (ACC), and to the site of discovery, such as the Miriam Hospital in Providence, R.I. (MIR-1) or Dhahran hospital in Saudi Arabia (DHA). BIL-1 was even named after the patient (Bilal) who provided the original sample (D. J. Payne, personal communication).

Increasingly, a demonstration of enzyme uniqueness relies on nucleotide sequencing, a sometimes imperfect technique. Barlow and Hall (M. Barlow and B. G. Hall, submitted for publication) recently reanalyzed several plasmid-mediated AmpC genes and concluded that the amino acid sequences of CMY-2, BIL-1, and LAT-2 were identical, as were LAT-1 and LAT-4 and also LAT-3 and CMY-6. In the text and tables below, the originally published names have been retained, with apparent identities in parentheses, such as BIL-1 (CMY-2).

EPIDEMIOLOGICAL FEATURES

As shown in Table 1, plasmid-mediated class C β-lactamases have been discovered most frequently in isolates of *K. pneumoniae* and also in other naturally AmpC$^-$ species such as *K. oxytoca*, *Salmonella*, and *P. mirabilis*. Some enzymes have been found in *E. coli*, although this species can also increase production of its normally weakly expressed chromosomal AmpC enzyme by gene duplication or mutation in the *ampC* promoter or attenuator with consequent enhanced gene expression (18, 62). In Greece, plasmid-mediated LAT-2 (CMY-2) has been found in clinical isolates of *Enterobacter aerogenes* simultaneously with its appearance in clinical strains of *K. pneumoniae* and *E. coli* (28), and in France plasmid-mediated ACC-1 has been found in both *E. coli* and *P. mirabilis* isolates obtained from the same urine sample (30). In the United States, ceftriaxone-resistant *Salmonella* have begun to appear that owe their resistance to plasmid-mediated CMY-2 β-lactamase. Between 1996 and 1998, 13 ceftriaxone-resistant *Salmonella* strains were isolated from symptomatic patients in eight different states (22). Several *Salmonella* serotypes were involved. Such strains have been isolated from cattle and pigs (87). One 12-year old boy living in Nebraska was infected with a ceftriaxone-resistant strain identical to *S. enterica* serotype Typhimurium strains isolated from his father's infected calves (23).

A striking feature is the global distribution of strains producing plasmid-determined cephalosporinases. They have been found in Africa (Algeria, Tunisia), Asia (India, Japan, Pakistan, South Korea), Europe (France, Germany, Greece, Italy, Sweden, United Kingdom), the Middle East (Saudi Arabia), North America (United States), and South and Central

America (Argentina, Guatemala) (Table 1). Just as with strains producing ESBLs such as SHV-2 or SHV-5 (77), travel and transfer of patients has allowed the importation of BIL-1 (CMY-2) from Pakistan to the United Kingdom (66), strains with BIL-1-like enzymes from the Indian subcontinent to London (59), several CMY types (CMY-2, CMY-6, CMY-7) from Punjab (India) to London (unpublished results), CMY-2 from Algeria to France (46), CMY-4 from India to Sweden (17), FOX-2 from Guatemala to Germany (10), ACC-1 from Tunisia to France (30, 60), and MOX-2 from Greece to France (S. Boyer-Mariotte, L. Raskine, B. Hanau, A. Philippon, M. M. Sanson-LePors, and G. Arlet, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-7, 1998). CMY-2 is the most prevalent of the plasmid-mediated AmpC enzymes and the most widely distributed geographically, having been reported in Algeria, France, Germany, Greece, India, Pakistan, Taiwan, Turkey, the United Kingdom, and the United States (5).

Except for *Salmonella* strains and occasional *K. pneumoniae* isolates (43), most strains producing plasmid-determined AmpC enzymes have been isolated from patients after several days in the hospital. Affected patients have often had prolonged stays in intensive care units. Some patients had one or more surgical procedures (65), an underlying disease such as leukemia (78) or cancer (27, 36, 59), or were immunocompromised after liver or kidney transplantation (59). Sources of organisms included cultures of urine (about 50% of isolates), blood, wounds, sputum, or stool. Some isolates were recovered in mixed cultures with other potential pathogens. A majority of the patients had been treated with β-lactam antibiotics including cefoxitin, moxalactam, cefmetazole, cefotetan, or imipenem (13, 31, 36, 65). Many strains with plasmid-determined AmpC enzymes also produce TEM-1, TEM-2, or even an ESBL, such as SHV-5 (28, 29, 59).

As already observed for ESBL-producing *K. pneumoniae* organisms (15, 61, 72), AmpC-producing nosocomial isolates can be responsible for outbreaks, for example, MIR-1 (11 patients) (65), a BIL-1(CMY-2)-like enzyme (5 patients) (59), ACC-1 (13 patients) (60), and ACT-1 (17 patients) (13).

SUSCEPTIBILITY PATTERNS

Table 2 illustrates in vitro susceptibilities for *E. coli* transconjugants or transformants producing plasmid-mediated AmpC β-lactamases. Some of the MICs for what ought to be similar enzymes differ by more than can be attributed to experimental variation (e.g., LAT-1 and LAT-4 with cefotaxime, ceftazidime, or aztreonam) and may have resulted from differences in host *E. coli* strains. Strains with plasmid-mediated AmpC enzymes were consistently resistant to aminopenicillins (ampicillin or amoxicillin), carboxypenicillins (carbenicillin or ticarcillin), and ureidopenicillins (piperacillin) and, among the penicillins, these strains were susceptible only to amdinocillin or temocillin. The enzymes provided resistance to cephalosporins in the oxyimino group (ceftazidime, cefotaxime, ceftriaxone, ceftizoxime, cefuroxime) and the 7-α-methoxy group (cefoxitin, cefotetan, cefmetazole, moxalactam). MICs were usually higher for ceftazidime than for cefotaxime and for cefoxitin than for cefotetan. The enzymes were also active against the monobactam aztreonam, although for some strains

TABLE 2. In vitro susceptibilities (MIC) of *E. coli* derivatives producing plasmid-encoded AmpC β-lactamase

Antimicrobial agent	MIC (μg/ml) (reference no.) for derivatives producing:																		
	ACC-1 (7)	ACT-1 (13)	BIL-1 (CMY-2)[a] (66, 88)	CMY-1 (9)	CMY-2 (8)	CMY-3 (14)	CMY-4 (85)	CMY-5 (90)	CMY-8 (91)	DHA-1 (26)	DHA-2 (24)	FOX-1 (31)	FOX-2 (10)	FOX-3 (51)	FOX-4 (12)	LAT-1 (83)	LAT-2 (CMY-2)[a] (28)	LAT-3 (29)	LAT-4 (LAT-1)[a] (29)	MIR-1 (65)	MOX-1 (36)
Ampicillin[b]			64	2,048		1,024	>128		>256	>512	>512		1,024	256	>128				1,000	>512
Carbenicillin[c]			128			1,024	>128			128	128	>2,048	≥1,024	512	>128				128		
Piperacillin	32	32	64	128	64		64			128	64			16						64	
Temocillin	4			8	8					4			8							4	
Mecillinam																					
Cephalothin[d]			>1,028		>1,024	>128				512	128									>512	
Cefotaxime	8	≤2	8	64	16	16	16	64	>256	64	4	2	16	1	64	128	64	96	4	64	>512
Ceftazidime	32	4	>16	4	128	64	8	256	32–64	64	8	8	32	16	>128	>128	>256	128	8	128	16
Cefoxitin	4	>256		256	256	128	8		>256	128	16	128	256	64	>512	64	256	256	64	≥256	
Cefotetan	2	16		256	64		32					32	64		128	128				≥64	>512
Cefmetazole				128	64							4								512	
Moxalactam	1			8	2		0.06			0.5		1	1		32					64	>512
Aztreonam	1	4	4	16	64	32		64		16	2	1	2	1	64	64	64	64	8	128	16
Cefepime	0.25	≤0.06		0.25	0.5		0.06			0.125	0.03		0.13	≤0.06	2			0.5	0.125	1	
Cefpirome	1			2	0.5						0.25		1							1	
Imipenem	0.13	1		0.25	0.5	0.25	0.25–1	0.25–0.5	≤0.125	0.25			0.5	0.12	0.5	2				1	0.5
Meropenem	0.03			0.06	0.06								0.03		0.12					0.125	

[a] Revised identity is shown in parentheses.
[b] Or amoxicillin.
[c] Or ticarcillin.
[d] Or cefazolin.

TABLE 3. Effect of β-lactamase inhibitors (MIC) on *E. coli* derivatives producing plasmid-mediated AmpC β-lactamases

Agent(s)	MIC (μg/ml) (reference no.) for derivatives producing:												
	ACC-1[a] (60)	BIL-1[b] (CMY-2)[h] (88)	CMY-1[c] (9)	CMY-2[c] (8)	CMY-3[b] (14)	DHA-1[d] (26)	DHA-2[a] (24)	FOX-1[e] (31)	FOX-2[b] (10)	FOX-3[d] (51)	FOX-4[d] (12)	LAT-1 (81, 83)	MIR-1[d] (65, 81)
Ampicillin or amoxicillin	>128	64	2,048		1,024	>512	>512			1,024	256	>128	1,000
+ Clavulanate	>128	>32	128		1,024	>512	>512			32	256	>64[g]	≥256
Cefoxitin			256	256				128	256	64	>512		
+ Clavulanate			64	128				64	128	64	>512		
+ Sulbactam			32	32					32				
+ Tazobactam			64	64					128				
Cefotaxime		8	64							1	64		
+ Clavulanate		>4	16							1	64		
+ Sulbactam			16										
+ Tazobactam			32										
Ceftazidime		>16	4				8			16	>128	32	128
+ Clavulanate		>4	4				8			16	>128	32[d]	128
+ Tazobactam												4[d]	16
+ Ro 48-1220												0.5[d]	2
Piperacillin	32		128	64			64						
+ Tazobactam	8		32	16			4						
Ticarcillin	256				1,024					≥1,024		>256	
+ Clavulanate	128				512					32		>128[g]	

[a] Clavulanate at 2 μg/ml and tazobactam at 4 μg/ml.
[b] Inhibitor concentration, 2 μg/ml.
[c] In the proportions of 1:4 (clavulanate), 1:1 (sulbactam), and 1:7 (tazobactam).
[d] Inhibitor concentration, 4 μg/ml.
[e] Inhibitor concentration, 5 μg/ml.
[f] In the proportion of 1:4 (clavulanate), 1:2 (sulbactam), and 1:8 (tazobactam).
[g] Clavulanate at 2 μg/ml with ticarcillin and in the proportion of 1:2 with ampicillin.
[h] Revised identity is shown in parentheses.

aztreonam MICs were in the susceptible range. Susceptibility to cefepime or cefpirome was little affected and was unchanged for carbapenems (imipenem, meropenem).

Alterations in antibiotic access to the enzyme can markedly change the susceptibility profile. With loss of outer membrane porin channels, *K. pneumoniae* strains carrying plasmids determining AmpC enzymes can have imipenem MICs as high as 64 μg/ml and meropenem MICs of 16 μg/ml (53). In such strains, MICs of cefepime and cefpirome become inoculum dependent, and at inocula of 10^7/ml MICs can exceed 256 μg/ml (53). Clinical isolates of *K. pneumoniae* and *E. coli* with imipenem MICs from 16 to 64 μg/ml through porin loss and carriage of ACT-1 (13) or CMY-4 (17, 78) have already been reported and are a cause for concern.

The effects of β-lactamase inhibitors on susceptibility is summarized in Table 3. Although commercially available inhibitors, especially sulbactam, caused a modest reduction in MICs of cefoxitin, *E. coli* derivatives with plasmid-mediated AmpC enzymes were not susceptible to inhibitor combinations, with the possible exception of piperacillin-tazobactam. Resistance was, however, blocked by BRL 42715 (7, 19, 53), Ro 47-8284 (7), Ro 48-1256 (50), or Ro 48-1220 (81).

TABLE 4. Substrate profile (V_{max} relative to cephaloridine) of plasmid-determined AmpC β-lactamases

Agent	V_{max} (reference no.)												
	ACC-1 (7)	ACT-1 (13)	CMY-3 (14)	DHA-1 (26)	FOX-1[c] (31)	FOX-3 (51)	FOX-4 (12)	LAT-1 (83)	LAT-2 (CMY-2)[d] (28)	LAT-3 (29)	LAT-4 (LAT-1)[d] (29)	MIR-1 (65)	MOX-1 (36)
Benzylpenicillin		7.1	5	67	1.3	1.2		5	3	4	5	4	
Ampicillin[a]			1	3			0.2	1	1	2	2	1	40
Carbenicillin[b]			<0.1					<1				<1	
Cephalothin			110	262	371	320		130	169	138	140	122	
Cefoxitin	<0.01	0.11	<0.1		0.74	<1	3.3	<1				<1	
Cefotetan	<0.01	0.014											8.3
Ceftazidime	≤0.1		<0.1	2		<1	0.25	1	2.2	2	2	3	1.5
Cefotaxime	<0.02	0.006	<0.1	<1		<1	0.09	<1	1	<1	<1	10	201
Moxalactam			<0.1	<1									2.4
Aztreonam		<0.1	<0.1	<1		<1							80

[a] Or amoxicillin.
[b] Or ticarcillin.
[c] Values shown are for pI 6.8 variant. pI 7.2 variant gave similar results.
[d] Revised identity is shown in parentheses.

TABLE 5. Affinity constants (K_m) of plasmid-encoded AmpC β-lactamases

Agent	K_m (μM) (reference no.)								
	ACC-1 (7)	ACT-1 (13)	BIL-1[a] (CMY-2)[c] (66)	CMY-3 (14)	FOX-1[b] (31)	FOX-3 (51)	FOX-4 (12)	LAT-2 (CMY-2)[c] (28)	MOX-1 (36)
Benzylpenicillin		10		3	43	36		12	
Ampicillin			8.7				23	17	2.4
Cephalothin				40	224	187		55	
Cephaloridine	122	380		90	375	363	1,400	10	134
Cefoxitin		3.7		0.2	1.3		37		
Cefotetan		2.5							805
Cefotaxime		7	0.01	0.5			1.2		1,064
Ceftazidime	17		20	115			14		2.7
Moxalactam				0.005					1.7
Aztreonam				0.01					40
Cefepime		1,040							
Imipenem		7.5							

[a] K_i values with nitrocefin as substrate.
[b] Values shown are for pI 6.8 variant; pI 7.2 variant gave similar results.
[c] Revised identity is shown in parentheses.

DETECTION

In bacterial species without an inducible AmpC-type β-lactamase, appearance of a susceptibility pattern typical of an *Enterobacter* or *C. freundii* isolate that overproduces its chromosomal β-lactamase is highly suggestive of a plasmid-encoded AmpC-type enzyme (56, 67). Unfortunately, other mechanisms can produce a similar resistance phenotype. In *E. coli* hyperproduction of chromosomal AmpC together with OmpF porin loss (52), or in *K. pneumoniae* porin deficiency alone (33), can give cephamycin and oxyimino-β-lactam resistance. Furthermore, not all strains with plasmid-mediated AmpC enzymes meet the NCCLS criteria for resistance to cephamycins and oxyimino-cephalosporins. For example, *E. coli* with ACC-1 can be resistant to ceftazidime but not cefoxitin or cefotetan, while a strain with DHA-2 was intermediate in resistance to cefoxitin but susceptible to cefotaxime or ceftazidime (Table 2). Suspected isolates can be studied further for cephamycin hydrolysis with the three-dimensional test (20, 80) or the Masuda bioassay (65) and for β-lactamase inhibitor effects. Unfortunately, inhibitors (BRL 42715, Ro 47-8284, Ro 48-1220, Ro 48-1256) that are active against AmpC enzymes are not readily available, but cloxacillin (69) or cefoxitin (65) have been used to block AmpC activity selectively after isoelectric focusing. Lack of inhibition of activity against oxyimino-β-lactams or cephamycins by clavulanate is indirect evidence for the presence of an AmpC enzyme, but some AmpC enzymes are unusually susceptible to inhibition by tazobactam (1). A reference laboratory is needed for β-lactamase isoelectric focusing or gene localization. Detecting a plasmid-mediated AmpC enzyme in a strain with a native inducible β-lactamase or a coexisting ESBL is even more challenging.

Given the difficulty in detecting plasmid-mediated AmpC β-lactamases, their prevalence is currently likely to be underestimated. Coudron et al. studied 1,286 consecutive, nonrepeat isolates from a single VA Medical Center collected between 1995 and 1997 and estimated that 1.6% of *E. coli* isolates, 1.1% of *K. pneumoniae* isolates, and 0.4% of *P. mirabilis* isolates were cefoxitin-resistant AmpC β-lactamase producers, mostly via transmissible plasmids (20).

ENZYMATIC PROPERTIES

Plasmid-mediated AmpC-type β-lactamases have pIs between 6.4 and 9.4 (Table 1). Several of the FOX-type enzymes show multiple satellite bands (31). In clinical isolates containing several β-lactamases, AmpC enzymes can be identified after isoelectric focusing by differential inhibition of nitrocefin reactivity with 5 mg of cefoxitin/ml (65) or detection of cefoxitin hydrolysis by bioassay (10). A few plasmids carry *ampR* as

TABLE 6. Inhibition profiles of plasmid-determined AmpC β-lactamases

Agent	50% inhibitory concn (μM) (reference no.)											
	ACT-1 (13)	BIL-1 (CMY-2)[b] (66)	CMY-3[a] (14)	DHA-1 (26)	FOX-1 (31)	FOX-3 (51)	LAT-1 (28, 81, 83)	LAT-2 (CMY-2)[b] (28)	LAT-3 (29)	LAT-4 (LAT-1)[b] (29)	MIR-1 (65)	MOX-1[a] (36)
Aztreonam				0.06	0.020	0.0015	0.03	0.02				
Cefoxitin		4.1					0.8	0.65			0.006	
Clavulanate	52	362	140	>100	>1	3	>250	250	>100	>100	0.21	5.6
Cloxacillin				0.1	0.023	0.02	0.001					0.35
Ro 48–1220							0.8		0.7	0.8		
Sulbactam	2.6	18	10						48	50		
Tazobactam	1.3	3.2	10	0.6			13		12	12	0.008	
Ticarcillin				0.01								

[a] Value shown is K_i instead of 50% inhibitory concentration.
[b] Revised identity is shown in parentheses.

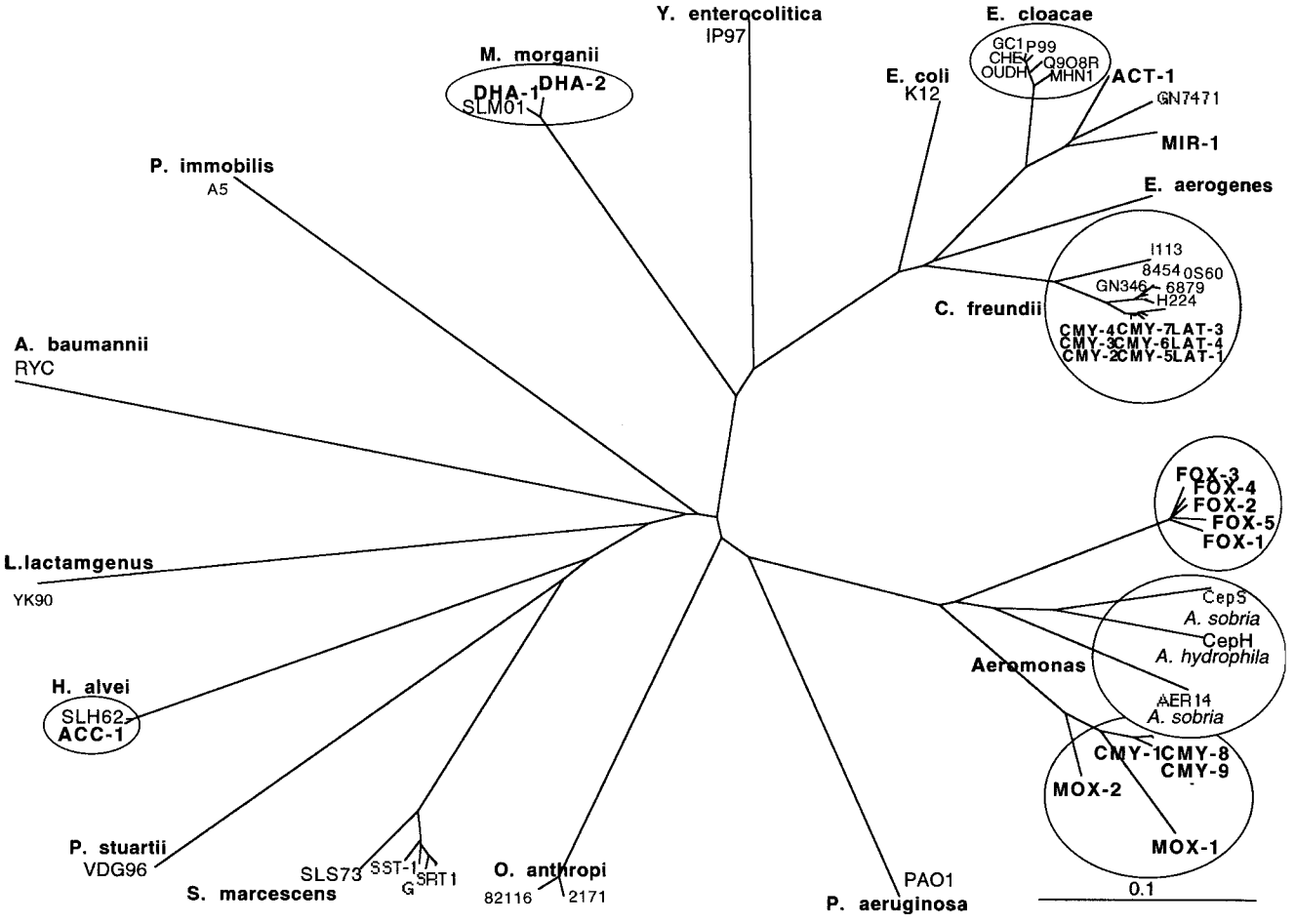

FIG. 1. Dendrogram for chromosomal and plasmid-encoded AmpC β-lactamases, calculated by Clustal X (34) and the neighbor-joining method (73). Branch lengths are proportional to the number of amino acid exchanges. GenBank accession numbers for bla_{AmpC} can be found at http://www.rochester.edu/College/BIO/labs/HallLab/AmpC_Phylo.html.

well as *ampC* genes and are inducible (DHA-1 and DHA-2) (3, 24), but most plasmid-mediated *ampC* genes are expressed constitutively, for example, MIR-1, even in the presence of a complete system for induction (41).

The apparent molecular size of the mature plasmid-mediated AmpC β-lactamases vary from 38 to 42 kDa with 378 (3), 381 (8, 29, 41), 382 (10, 37), or 386 (7) amino acid residues. Kinetic properties were characteristic of chromosomal AmpC enzymes with relative V_{max} values generally 10-fold or more greater for cephalothin and cephaloridine than for ampicillin and penicillin, greater activity with penicillin than with ampicillin, and low hydrolysis rates for oxyimino- or α-methoxy-compounds (Table 4). On the other hand, K_m values for cefoxitin, cefotetan, cefotaxime, moxalactam, or aztreonam were generally less than those for penicillin or ampicillin and much lower than the K_m values for cephaloridine, cephalothin, or cefepime (Table 5).

As is typical of group 1 cephalosporinases (16), plasmid-mediated AmpC enzymes were inhibited by low concentrations of aztreonam, cefoxitin, or cloxacillin and only by high concentrations of clavulanate (Table 6). Sulbactam and particularly tazobactam were more effective inhibitors but were an order of magnitude less potent than Ro 48-1220.

The amino acid sequence of the enzymes revealed an active-site serine in the motif Ser-X-X-Lys (where X is any amino acid) at residues 64 to 67 of the mature protein. A Lys-Ser/Thr-Gly motif has been found at residues 315 to 317 and plays an essential role in forming the tertiary structure of the active site. A tyrosine residue at position 150 forms part of the class C-typical motif Tyr-X-Asn and is also important (but not essential) for catalysis of β-lactam hydrolysis (21, 64)

A dendrogram of chromosomal and plasmid-encoded AmpC enzymes (Fig. 1) demonstrates the diversity of chromosomal AmpC genes and the close relationship of some plasmid-mediated enzymes to chromosomal enzymes of particular organisms. The plasmid-mediated enzymes can be divided into five or six clusters: the *C. freundii* group with LAT types and certain CMY types, the *Enterobacter* group with MIR-1 and ACT-1, the *M. morganii* group with DHA-1 and DHA-2, the *H. alvei* group represented by ACC-1, and the *Aeromonas* group with MOX-, FOX-, and other CMY-type enzymes. The relationship between plasmid-encoded enzymes and certain chromosomal β-lactamases is very close: there is 100% amino acid homology within the *M. morganii* and *H. alvei* groups and more than 94% homology within the *C. freundii* group. ACT-1 and MIR-1 share 91.4% amino acid identity with each other but

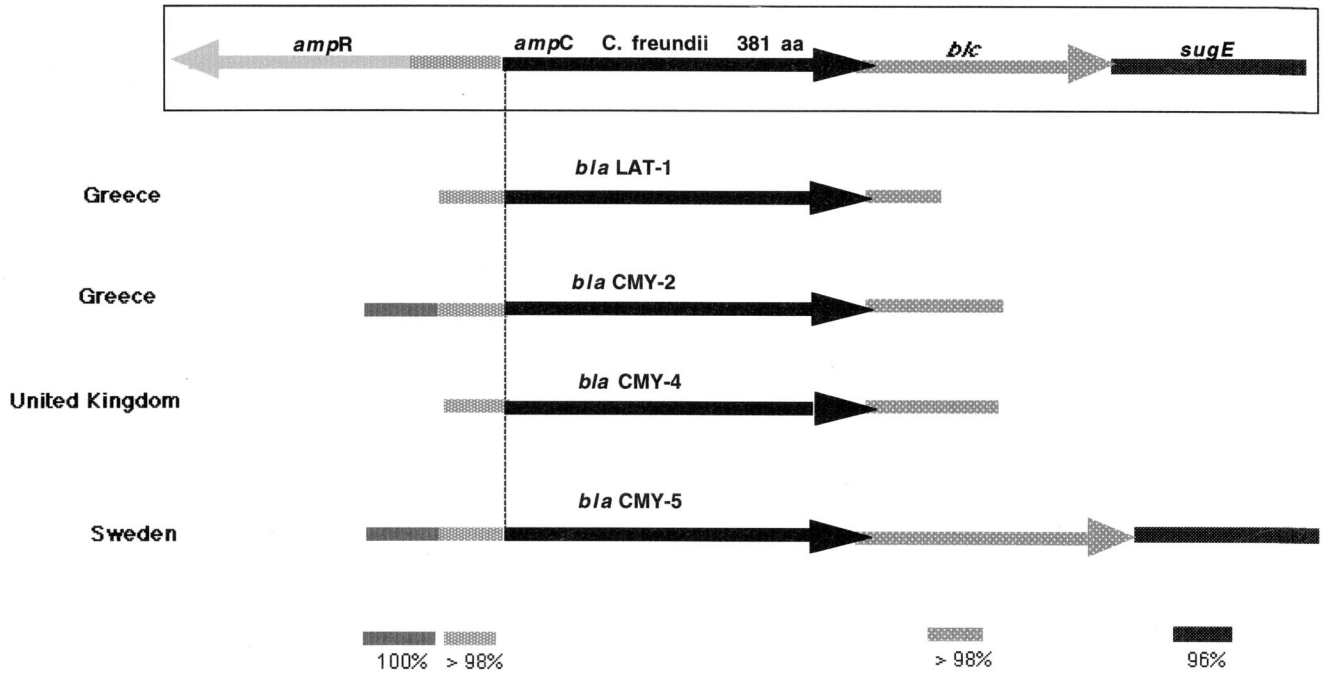

FIG. 2. Comparative genetic organization of plasmid-encoded *ampC* genes related to the chromosomal β-lactamase gene of *C. freundii*. Adjacent nucleotide homology is that available for each gene and may be more extensive if carried further.

only 85 to 87% identity with most *E. cloacae* AmpC enzymes. However, the enzyme from *E. cloacae* strain GN7471 (47) has 91.1% identity to ACT-1 and MIR-1, and an environmental strain of *Enterobacter* was 98% identical (M. Rottman and G. Arlet, personal communication), so that origin from some *Enterobacter* species is likely. FOX enzymes have 95% or more sequence identity within the group, and CMY-1, CMY-8, and CMY-9 are more than 97% identical, but either group has only about 74% identity with the available *Aeromonas sobria* AmpC sequences, which in turn differ from each other by fully 25%. The AmpC sequence of *P. aeruginosa* is even more distant, so the origin of these enzymes remains uncertain.

Unlike ESBLs, which differ from their parent enzymes by amino acid substitutions that alter the properties of the active site (45), remodeling seems not to be necessary for the success of a plasmid-mediated AmpC enzyme. M. Barlow and B. G. Hall (submitted) found that chromosomal *ampC* genes from *C. freundii* strains isolated in the 1920s were just as effective in providing β-lactam resistance as plasmid-mediated *ampC* alleles such as CMY-2 or LAT-3 when all were cloned into a common vector in a common *E. coli* host. Nonetheless, once a bla_{AmpC} gene is plasmid-borne further evolution may occur. For example, a naturally occurring Asn→Ile substitution at position 346 in CMY-1 has been reported to increase the ceftazidime MIC for a strain with the variant enzyme by four- to eightfold (A. Bauernfeind, I. Schneider, D. Meyer, R. Jungwirth, Y. Chong, and K. Lee, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-203, 1997). It is likely that in time other such changes will be observed, since a variety of genetic changes can improve the activity of the chromosomal AmpC enzyme of *E. cloacae* to particular substrates, such as activity toward cefuroxime and ceftazidime with a tandem duplication of three amino acids at positions 208 to 210 (63) or toward cefepime and cefpirome with an amino acid

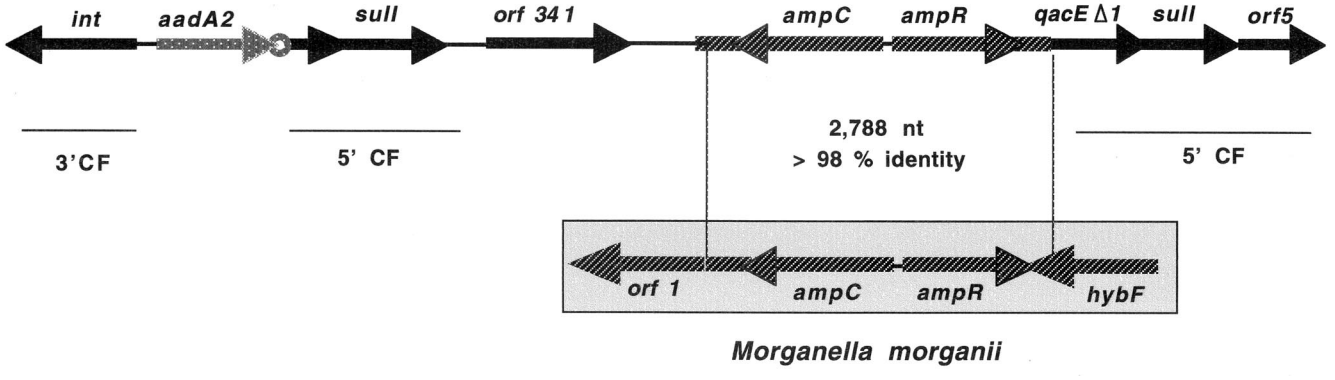

FIG. 3. Structure of the integron coding for DHA-1 β-lactamase in pSAL-1 and its relationship to genes on the chromosome of *M. morganii*.

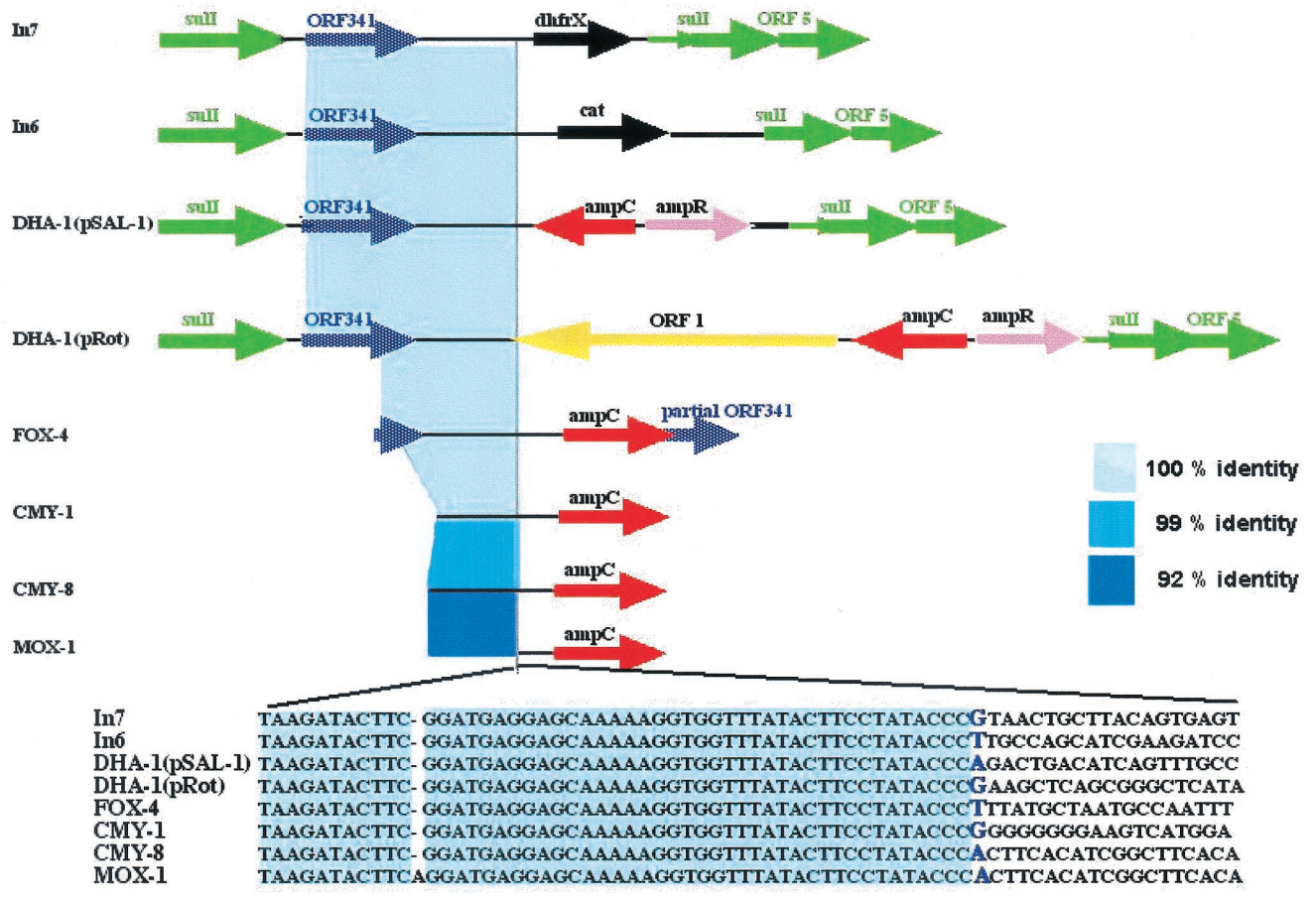

FIG. 4. Comparison of the sequence between common regions of In7, In6, pSAL-1 (DHA-1), pROT (DHA-1), pGC-2 (FOX-4), pMVP-1 (CMY-1), pKPW142 (CMY-8), and pRMOX-1 (MOX-1).

substitution at position 318 (58) or a deletion of six amino acids at residues 289 to 294 (4).

GENETIC FEATURES

Genes for the AmpC enzymes have been located on plasmids of sizes varying from 7 to 180 kb (37, 78). A few of the plasmids have not been self-transmissible but are transferable by transformation (65, 78, 89) or mobilization (28, 29, 82, 83). Plasmids encoding AmpC enzymes often carry multiple other resistances, including resistance to aminoglycosides, chloramphenicol, sulfonamide, tetracycline, trimethoprim, or mercuric ion (9, 10, 13, 65, 78). A plasmid encoding a FOX-type enzyme even carried a gene for fluoroquinolone resistance (54). Clinical isolates often produce other β-lactamases in addition to an AmpC enzyme. The *bla* genes may be on different plasmids, but often they coexist on the same plasmid. For example, the gene for ACT-1 was found in clinical isolates along with a pI 5.6 β-lactamase consistent with TEM-10 or TEM-26 and a pI 7.6 enzyme consistent with SHV-1, and on cloning the ACT-1 gene was found in a 15-kb cluster with genes for a β-lactamase of pI 5.4, presumably TEM-1, and pI 7.0, possibly another SHV-type enzyme (13). Furthermore, an ACT-1 probe hybridized to chromosomal DNA of these clinical strains, implying mobility of the gene by carriage on a transposon. Indirect evidence suggests that CMY-3 and CMY-4 could be transposon-mediated as well: CMY-3 because its gene is located on the chromosome of a species (*P. mirabilis*) lacking a native AmpC gene (14), and CMY-4 because in *E. coli* clinical isolates from London a CMY-4 probe hybridized to both 7-kb and 45-kb plasmids, a dual location that could be explained by transposability (78). The MIR-1 gene is located near a sequence closely related to an insertion sequence transposase, but direct attempts to demonstrate transposability of MIR-1 or BIL-1 (CMY-2) have not been successful (25, 41).

The *C. freundii*-type bla_{CMY-5} gene has been mapped in plasmid pTKH11 to be adjacent to the *blc* and *sugE* genes found downstream from *ampC* on the *C. freundii* chromosome (89). The *ampR* gene upstream from *ampC* on the chromosome is missing and its place has been taken by a putative insertion element which could have been involved in gene capture. So far as they have been sequenced, other plasmids encoding *C. freundii*-type AmpC enzymes have a similar organization (Fig. 2), suggesting a direct derivation from the *C. freundii* chromosome with subsequent accumulation of mutations in the *ampC* gene to produce the present array of CMY- and LAT-type enzymes, a conclusion also supported by phylogenetic analysis (M. Barlow and B. G. Hall, submitted).

Many resistance genes, including those for Ambler class A, B, and D β-lactamases, are located in gene cassettes with a downstream 59-base element that acts as a specific recombi-

nation site for incorporation into integrons (32). Analysis of published sequences indicates that AmpC genes found on plasmids are not linked to 59-base elements. The DHA-1 structural and regulatory genes on plasmid pSAL-1, however, are present in an integron (Fig. 3) which includes a site-specific integrase, two copies of *qacEΔ1sulI*, an *aadA2* gene for aminoglycoside resistance with its downstream 59-base element, and ORF341, a postulated recombinase (84). The genetic organization of this integron is similar to In6 and In7 found in plasmids pSa and pDGO100, which lack *bla* genes (79). *ampC* and *ampR* genes from the chromosome of *M. morganii* (2, 70) thus appear to have inserted into a complex *sulI*-type integron. Since no downstream 59-base element remains, either it was deleted or integration involved another mechanism. Verdet et al. identified a 50-bp sequence near bla_{DHA-1} that is present also in In6 and In7. A virtually identical sequence occurs near genes for FOX-4, CMY-1, CMY-8, and MOX-1 (Fig. 4). Such a sequence could represent a recombination site different from the 59-base element. Further study of the structure of plasmids carrying *ampC* genes is likely to reveal additional tricks used by bacteria to mobilize genes for dissemination.

CONCLUSIONS

Among gram-negative bacteria, the emergence of resistance to expanded-spectrum cephalosporins has been a major concern, initially in a limited number of bacterial species (*E. cloacae*, *C. freundii*, *S. marcescens*, and *P. aeruginosa*) that could mutate to hyperproduce their chromosomal class C β-lactamase. A few years later, resistance appeared in bacterial species not naturally producing AmpC enzymes (*K. pneumoniae*, *Salmonella* spp., *P. mirabilis*) due to the production of TEM- or SHV-type ESBLs. Characteristically, such resistance has included oxyimino- but not 7-α-methoxy-cephalosporins, has been blocked by inhibitors such as clavulanate, sulbactam, or tazobactam, and did not involve carbapenems. Plasmid-mediated AmpC β-lactamases represent a new threat since they confer resistance to 7-α-methoxy-cephalosporins such as cefoxitin or cefotetan, are not affected by commercially available β-lactamase inhibitors, and can, in strains with loss of outer membrane porins, provide resistance to carbapenems. This resistance mechanism has been found around the world, can cause nosocomial outbreaks, appears to be increasing in prevalence, and merits further study to define the best options for detection and treatment.

ADDENDUM

The sequence of ORF341 has recently been revised, extending the open reading frame to 1,541 nucleotides that encode a protein of 513 amino acids; this sequence has been renamed ORF 513 (GenBank accession no. L06418).

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