Activity of Ertapenem (MK-0826) versus *Enterobacteriaceae* with Potent β -Lactamases

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Ertapenem (MK-0826; L-749,345), a new carbapenem with a long serum half-life, was tested, *in vitro*, against β -lactamase-producing bacteria. The new compound had a MIC at which 90% of the isolates were inhibited of 0.06 μ g/ml for extended-spectrum β -lactamase (ESBL)-producing klebsiellas, compared with 0.5 μ g/ml for imipenem, 16 μ g/ml for cefepime, and >128 μ g/ml for ceftazidime and piperacillin-tazobactam. MICs of ertapenem for AmpC-derepressed mutant *Enterobacteriaceae* were 0.015 to 0.5 μ g/ml, whereas imipenem MICs were 0.25 to 1 μ g/ml and those of cefepime were 0.5 to 4 μ g/ml, and resistance to ceftazidime and piperacillin-tazobactam was generalized. Despite this good activity, the MICs of ertapenem for ESBL-positive klebsiellas mostly were two- to fourfold above those for ESBL-negative strains, and the MICs for AmpC-hyperproducing *Enterobacter cloacae* and *Citrobacter freundii* mutants exceeded those for the corresponding AmpC-basal mutants. These differentials did not increase when the inoculum was raised from 10^4 to 10^6 CFU/spot, contra-indicating significant lability. Carbapenemase producers were also tested. The IMP-1 metallo- β -lactamase conferred substantial ertapenem resistance (MIC, 128 μ g/ml) in a porin-deficient *Klebsiella pneumoniae* strain, whereas a MIC of 6 μ g/ml was recorded for its porin-expressing revertant. SME-1 carbapenemase was associated with an ertapenem MIC of 2 μ g/ml for *Serratia marcescens* S6, compared with <0.03 μ g/ml for *Serratia* strains lacking this enzyme. In summary, ertapenem had good activity against strains with potent β -lactamases, except for those with known carbapenemases.

Imipenem and meropenem retain activity against bacteria with extended-spectrum β -lactamases (ESBLs) and those that hyperproduce AmpC type β -lactamases and against *Klebsiella oxytoca* strains that hyperproduce the K1 enzyme (16). They also retain activity against strains with many less-frequent cephalosporinases, such as the PER and CTX-M enzymes. This activity gives these carbapenem drugs major advantages over cephalosporins, since potent β -lactamases are becoming increasingly prevalent. In recent surveys, ESBLs were found in 23 to 25% of klebsiellas from European intensive care units (2, 19); moreover, *Klebsiella* strains with multiple ESBLs are increasingly seen (10). AmpC β -lactamases are chromosomal and ubiquitous in *Enterobacter* spp., *Citrobacter freundii*, *Morganella morganii*, and *Serratia* spp., where they give resistance to oxyimino-aminothiazolyl cephalosporins if they are hyperproduced as a consequence of mutation (16); in addition AmpC genes can escape to plasmids, spreading resistance into further species (20). Hyperproduction of the K1 enzyme is seen in an increasing proportion of *K. oxytoca* isolates in European intensive care units (2, 11).

The stability of imipenem and meropenem in the presence of potent β -lactamases makes the carbapenems an attractive class for further development (9). Ertapenem (MK-0826, L-749,345) (15) is a new analogue in the class, notable for a

long serum half-life (ca. 4 h). We evaluated its activity against strains with potent cephalosporin-hydrolyzing β -lactamases and against *Enterobacteriaceae* with class A and B β -lactamases that confer resistance to imipenem. The latter enzymes are rare and largely restricted to nonfermenters but may represent a future problem in *Enterobacteriaceae* too (17, 18).

MATERIALS AND METHODS

Bacteria. The *Klebsiella pneumoniae* and *K. oxytoca* isolates were collected from intensive care unit patients in western and southern Europe during a survey undertaken in 1994 (19). They included 181 klebsiellas inferred to have ESBLs on the basis of ceftazidime MIC/ceftazidime plus clavulanate MIC ratios ≥ 16 . SHV-derived ESBLs in these isolates were typed by isoelectric focusing and PCR-single-strand conformation polymorphism (27); TEM variants were typed by isoelectric focusing and PCR-restriction fragment length polymorphism (26). A few *bla*_{SHV} and *bla*_{TEM} genes were sequenced (26, 27). Also included, from the same survey (19), were seven *Klebsiella* isolates with AmpC β -lactamases and 19 *K. oxytoca* isolates that hyperproduced K1 β -lactamase. Twenty-five *K. oxytoca* and 25 *K. pneumoniae* isolates without potent β -lactamase types were included as controls; these were susceptible to extended-spectrum cephalosporins at ≤ 2 μ g/ml and gave ceftazidime MIC/ceftazidime plus clavulanate MIC ratios ≤ 4 .

To assess interactions with inducible and derepressed chromosomal β -lactamases, isogenic mutant series of *Enterobacter cloacae*, *C. freundii*, *Serratia marcescens*, *M. morganii*, and *Proteus vulgaris* were tested (3, 25). Most series comprised a β -lactamase-inducible isolate and its β -lactamase-derepressed and -basal mutants, but some lacked β -lactamase-inducible parent strains, having been derived from derepressed isolates. To assess the effect of carbapenemases, the antibiotics were tested against *S. marcescens* S6, with the SME-1 enzyme (class A) (21, 25), and against *K. pneumoniae* K4181, a clinical isolate from Singapore with the IMP-1 metallo- β -lactamase and loss of a 39-kDa porin (14). *Escherichia coli* ATCC 25922 was a general control.

Susceptibility tests. MICs were determined by National Committee for Clinical Laboratory Standards (NCCLS) agar dilution methodology (22). In some

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TABLE 1. MIC distributions for klebsiellas with different β -lactamase profiles

MIC (μg/ml)	No. of isolates ^a producing indicated β-lactamase when treated with ^b :														
	Ertapenem					Imipenem					Cefepime				
	ESBL	AmpC	K1	None (K pn control)	None (K oxy control)	ESBL	AmpC	K1	None (K pn control)	None (K oxy control)	ESBL	AmpC	K1	None (K pn control)	None (K oxy control)
0.007	8		3	20	1										
0.015	49	2	9	<u>4</u>	23										
0.03	71	2	3		2									9	18
0.06	39	3	<u>3</u>	1		14	1	5	1	1	5			13	<u>5</u>
0.12	9		1			125	5	5	17	14	3	2		<u>2</u>	<u>2</u>
0.25						22	1	10	<u>7</u>	<u>8</u>	7	2	6		
0.5	2					<u>15</u>		<u>4</u>		2	16	1	3	1	
1	1					4					40	1	3		
2	1					1					51		4		
4											36		<u>3</u>		
8	1										<u>13</u>	1			
16											8				
32											2				
64															
128															
256															
>256															

^a Values in boldface and those underlined and in boldface represent numbers of isolates for which the associated MICs are MIC₅₀s and MIC₉₀s, respectively. Where MIC₉₀ = MIC₅₀, the values are given MIC₉₀ labeling.

^b K pn, *K. pneumoniae*; K oxy, *K. oxytoca*.

experiments the inocula were adjusted to 10⁴ and 10⁶ CFU/spot to investigate inoculum effects. The drugs tested were ertapenem and imipenem (Merck, Rahway, N.J.), ceftazidime (GlaxoSmithKline, Stevenage, United Kingdom), cefepime (Bristol-Myers Squibb, Hounslow, United Kingdom), and piperacillin and piperacillin-tazobactam (Wyeth, Taplow, United Kingdom). Tazobactam was used at 4 μ g/ml. In a few experiments, MICs were redetermined with E-tests (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar, in accordance with the manufacturer's directions.

RESULTS

MICs for klebsiellas in relation to β -lactamase types. MIC distributions for the klebsiellas with ESBLs, AmpC enzymes, or hyperproduction of the K1 enzyme are illustrated in Table 1. The MIC at which 50% of the isolates were inhibited (MIC₅₀) and MIC₉₀ of ertapenem for the ESBL producers were 0.03 and 0.06 μ g/ml, respectively, and were lower than those for any isolate compared, with the new compound about four- to eightfold more active than imipenem and with both these carbapenems considerably more active than any other drug compared. Despite these favorable results, the MICs of ertapenem for ESBL producers were about fourfold above those for nonproducers, which were inhibited by ertapenem at 0.06 μ g/ml or less. MICs of ertapenem for three producers were 1 μ g/ml or more, although always less than 16 μ g/ml. MICs for these three isolates were redetermined with E-tests, which gave values 1 dilution below those found by agar dilution (not shown).

Imipenem had a MIC₅₀ of 0.125 μ g/ml for both ESBL producers and nonproducers and had a MIC₉₀ only 1 dilution higher for ESBL producers than for nonproducers (0.5 versus 0.25 μ g/ml). An imipenem MIC of 1 or 2 μ g/ml was recorded for five ESBL producers, whereas MICs for nonproducers were universally 0.5 μ g/ml or less. The MICs of the cephalosporins for ESBL producers and nonproducers were much

more stratified than were those of the carbapenems (Table 1). Thus, the MIC₅₀ of ceftazidime for ESBL producers was 64 μ g/ml, whereas that for nonproducers was 0.25 μ g/ml. For cefepime, the MIC₅₀ and MIC₉₀ for ESBL producers were 2 and 8 μ g/ml, respectively, compared with 0.06 and 0.12 μ g/ml for ESBL-negative *K. pneumoniae* and 0.03 and 0.06 μ g/ml for ESBL-negative *K. oxytoca*. MICs of piperacillin exceeded 64 μ g/ml for the huge majority (93%) of ESBL producers, whereas MICs for most nonproducers were from 4 to 16 μ g/ml. Tazobactam, at 4 μ g/ml, lowered the MICs of piperacillin for virtually all the isolates, ESBL producing or not. The distribution of MICs of piperacillin-tazobactam for ESBL producers was bimodal, with peaks at 8 and >256 μ g/ml and with 69.5% of the producers susceptible at the NCCLS breakpoint (22) of 16 plus 4 μ g/ml.

MICs for the 7 klebsiellas with AmpC enzymes are shown in Table 1, as are those for the 19 *K. oxytoca* isolates that hyperproduced the K1 enzyme. MICs of ertapenem for the AmpC producers were two- to fourfold higher than those for the control klebsiellas but remained $\leq$ 0.06 μ g/ml, whereas the MICs of imipenem for the AmpC producers were no higher than those for the control strains. Production of AmpC enzymes was associated with high-level resistance (MICs, mostly $\geq$ 32 mg/ml) to ceftazidime, piperacillin, and piperacillin-tazobactam and with MICs of cefepime raised to 0.125 to 0.5 μ g/ml, compared with 0.03 to 0.06 μ g/ml for control strains.

The MIC₅₀s of both carbapenems were raised by only about 1 dilution for *K. oxytoca* strains that hyperproduced the K1 β -lactamase: to 0.25 μ g/ml for imipenem and 0.015 μ g/ml for ertapenem. Hyperproduction of the K1 enzyme was associated with high-level resistance (MICs, mostly $\geq$ 64 μ g/ml) to piperacillin and piperacillin-tazobactam and with cefepime and ceftazidime MICs raised to 0.25 to 4 μ g/ml.

TABLE 1—Continued

No. of isolates ^a producing indicated β-lactamase when treated with ^b :														
Ceftazidime					Piperacillin					Piperacillin c-tazobactam				
ESBL	AmpC	K1	None (K pn control)	None (K oxy control)	ESBL	AmpC	K1	None (K pn control)	None (K oxy control)	ESBL	AmpC	K1	None (K pn control)	None (K oxy control)
1		6	1	10						1				
3	1	3	14	12										
4		3	8	2						1				
4		4	2										1	
2		3								14			10	1
9								3		30		2	9	11
11					1			5	11	58	1	1	4	11
15					3		2	16	10	22			1	1
24					2	1				10	3	2		
25	2				6	1				4		14		1
45	2				19	1			2	6	1			
38	2				39	1	17		1	7				
					111	3			1	28	2			

Activity against chromosomal β -lactamase expression mutants. MICs for AmpC β -lactamase inducibility variants of *E. cloacae*, *C. freundii*, *M. organii*, and *S. marcescens* are shown in Table 2, together with data for *P. vulgaris* mutants varying in expression of their class A chromosomal β -lactamase. Ertapenem MICs for AmpC-derepressed *E. cloacae* and *C. freundii* mutants were up to 128-fold above the exquisitely low values for the corresponding AmpC-basal mutants but never exceeded 0.5 $\mu\text{g/ml}$. AmpC-inducible *E. cloacae* and *C. freundii* mostly were more susceptible than their derepressed mutants but less susceptible than their β -lactamase-basal mutants, implying that inducible β -lactamase also gave slight protection against ertapenem. MICs of ertapenem for *M. organii*, *S. marcescens*, and *P. vulgaris* variants were unrelated to enzyme expression, being equal (± 1 dilution) for β -lactamase-inducible, -derepressed, and -basal organisms within a mutant series.

Imipenem MICs were unrelated to AmpC expression for all species, including *E. cloacae* and *C. freundii*. Derepression of AmpC raised the MICs of cefepime by up to 64-fold for *E. cloacae* and *C. freundii*, but the MICs for derepressed mutants never exceeded 2 µg/ml; derepression had little effect on the cefepime MICs for *S. marcescens*, *M. morganii*, and *P. vulgaris*. Derepression, but not inducible expression, was associated with resistance to piperacillin and, except in *S. marcescens*, to ceftazidime. Tazobactam, 4 µg/ml, reversed piperacillin resistance for derepressed *P. vulgaris* and *M. morganii* mutants, not for other species.

Inoculum effects. MICs were determined with inocula of 10^4 and 10^6 CFU/spot for selected ESBL-producing klebsiellas and controls and for AmpC-derepressed and -inducible *Enterobacteriaceae* (Table 3). The maximum inoculum effect with ertapenem for an ESBL producer was eightfold, and most effects were fourfold or less; effects of a similar magnitude were seen for the ESBL-negative klebsiellas used as controls. Inoculum effects with imipenem were slightly greater than those with ertapenem and, again, were unrelated to ESBL production. A

much greater differential was seen with cefepime, which typically had 8- to 128-fold inoculum effects for ESBL producers, compared with 2- to 4-fold effects for nonproducers. Most ESBL producers were resistant (MICs, $>16 \mu\text{g/ml}$) to piperacillin and ceftazidime even at low inocula, precluding calculation of meaningful inoculum effect ratios. The magnitude of the inoculum effects with piperacillin-tazobactam was highly variable. Uniquely among the compounds tested, piperacillin had substantial (8- to 16-fold) inoculum effects for the ESBL-negative klebsiellas, presumably because these have chromosomal LEN, SHV, or K1 enzymes, which are active against piperacillin (16; G. S. Babini and D. M. Livermore, Letter, *Antimicrob. Agents Chemother.* **44**:22302000).

The inoculum effects of ertapenem and imipenem for AmpC-derepressed strains were 2- to 4-fold, whereas ratios of 8- to 32-fold were seen for cefepime; AmpC-derepressed organisms mostly were resistant to piperacillin and ceftazidime at low inocula, precluding calculation of meaningful inoculum effect ratios. Inoculum effects for the AmpC-inducible control strains were widely scattered for all the compounds; this observation is subject to the caveat that the increased MICs with higher inocula may have reflected the selection of derepressed mutants, not growth of the majority population.

Activity against carbapenemase producers. *K. pneumoniae* K4181, with the IMP-1 enzyme and lacking a 39-kDa porin (14), was highly resistant (MIC, $>32 \mu\text{g/ml}$) to all the β -lactams tested, including both carbapenems (Table 4). The MICs of imipenem and ertapenem fell to 2 and 6 $\mu\text{g/ml}$, respectively, for a variant that retained the carbapenemase but that had regained expression of the porin. This variant remained highly resistant to the noncarbapenem drugs compared. *S. marcescens* S6, with the SME-1 enzyme, was resistant to imipenem (MIC, 32 $\mu\text{g/ml}$) and had reduced susceptibility to ertapenem (MIC, 2 $\mu\text{g/ml}$) compared with typical *S. marcescens* strains such as S2 and S7 in Table 2. This strain retained good susceptibility to the other drugs compared.

TABLE 2. MICs for chromosomal β -lactamase inducibility mutant strains of *Enterobacteriaceae*

Strain ^a	MIC (μ g/ml) of:					
	Ertapenem	Imipenem	Ceftazidime	Cefepime	Piperacillin	PIPTAZ ^b
<i>C. freundii</i>						
C2	0.03	0.5	0.5	0.06	8	4
C2-con	0.125	0.125	64	1	256	64
C2-def	0.007	0.125	0.5	0.06	8	2
C4	0.06	2	8	0.06	16	4
C4-con	0.125	0.25	128	0.5	256	64
C4-def	0.007	0.125	0.06	0.03	4	1
C10	0.007	0.125	0.5	0.03	8	4
C10-con	0.125	0.25	64	0.5	128	32
C10-def	0.004	0.25	0.25	0.015	2	2
C12	0.015	0.5	0.25	0.03	8	4
C12-con	0.25	0.125	64	0.5	128	32
C12-def	0.004	0.125	0.25	0.015	2	2
<i>E. cloacae</i>						
84-con	0.25	0.125	128	2	>256	128
84-def	0.015	0.125	2	0.06	16	8
684	0.06	0.125	0.5	0.06	8	4
684-con	0.5	0.25	128	2	256	64
684-def	0.004	0.125	0.25	0.06	4	4
410-con	0.125	0.5	32	0.25	64	16
410-def	0.007	0.125	0.5	0.125	4	4
100-con	0.015	0.125	16	0.125	128	8
100-def	0.007	0.125	0.25	0.03	1	1
<i>M. morgani</i>						
M1	0.015	2	0.06	0.015	1	0.25
M1-con	0.015	2	8	0.03	64	0.25
M1-def	0.007	1	0.06	0.015	0.5	0.25
M3-con	0.15	2	16	0.03	64	0.25
M3-def	0.15	4	0.125	0.06	2	0.05
M6	0.015	2	0.06	0.03	1	0.25
M6-con	0.007	2	8	0.015	32	0.25
M6-def	0.007	0.5	0.06	0.015	0.25	0.25
<i>S. marcescens</i>						
S2	0.03	1	0.25	0.125	4	4
S2-con	0.06	1	2	0.5	128	64
S2-def	0.015	0.5	0.25	0.125	4	4
S7	0.015	0.5	0.25	0.06	4	2
S7-con	0.03	0.5	1	0.5	128	64
S7-def	0.03	0.5	0.25	0.25	4	4
<i>P. vulgaris</i>						
Va1	0.015	1	0.125	0.125	4	1
Va1-con	0.015	0.25	0.125	0.125	32	1
Va1-def	0.007	0.125	0.06	0.06	1	0.25
V2	0.015	1	0.06	0.125	4	1
V2-con	0.007	0.25	0.25	0.5	64	0.5
V2-def	0.007	0.25	0.06	0.06	2	0.5
V3	0.007	1	0.06	0.06	2	1
V3-con	0.007	0.5	0.125	0.5	32	1
V3-def	0.007	0.25	0.06	0.06	0.5	0.5
V11	0.015	0.25	0.03	0.06	0.5	0.5
V11-con	0.015	0.25	0.03	0.06	16	0.5
V11-def	0.007	0.25	0.06	0.06	0.5	0.5
V17	0.015	0.25	0.06	0.06	1	0.5
V17-con	0.007	0.125	0.125	0.5	64	0.5
V17-def	0.004	0.06	0.03	0.03	0.5	0.25

^a Suffix con indicates strains that hyperproduced chromosomal β -lactamases independently of induction; suffix def indicates strains that had only trace levels of chromosomal β -lactamases; strains without suffixes are β -lactamase inducible.

^b PIPTAZ, piperacillin-tazobactam.

TABLE 3. Inoculum effects for ertapenem and drugs compared

Isolates (no. tested)	Antibiotic	No. of isolates with:										MIC > 16 µg/ ml at inoculum of 10 ⁴ CFU/spot
		Ratio of MICs at inocula of 10 ⁴ CFU/spot and 10 ⁶ CFU/spot ^a of:										
		<1	1	2	4	8	16	32	64	128	≥256	
Klebsiellas, ESBL producers (50)	Ertapenem		11	25	13	1						
	Imipenem		1	11	16	12	9	1				
	Ceftazidime		1	4		1						44
	Cefepime				1	8	16	9	10	5		1
	Piperacillin											50
	PIPTAZ			3	1	1	3	2	1	1		38
AmpC-derepressed <i>Enterobacteriaceae</i> and klebsiellas with plasmid- mediated AmpC enzymes (19) ^c	Ertapenem		2	8	5	4						
	Imipenem		3	8	6	1	1					
	Ceftazidime				2	2	1					14
	Cefepime			1	7	4	4	3				
	Piperacillin											19
	PIPTAZ		1	3	3	1	2	1				8
Klebsiella controls, cephalosporin susceptible (10)	Ertapenem		2	5	3							
	Imipenem		2	3	3	1	1					
	Ceftazidime	2	1	5	1			1				
	Cefepime		1	6	2			1				
	Piperacillin				1	3	3	2				1
	PIPTAZ	1	2	5	1							1
AmpC-inducible <i>Enterobacteriaceae</i> controls (9) ^d	Ertapenem			5	2		2					
	Imipenem		1		5	2	1					
	Ceftazidime		2	1	2			2	1			1
	Cefepime		2		3	2	1		1			
	Piperacillin			3	1		3	1				1
	PIPTAZ		2	2	1	2			1			1

^a Ratios were calculated only where the MIC was ≤ 16 μ g/ml with an inoculum of 10^4 CFU/spot. In some cases the MIC with an inoculum of 10^6 exceeded the highest drug concentration tested; in these instances the MIC was assumed to be 1 dilution higher. Thus, if the MICs with inocula of 10^4 and 10^6 CFU/spot were 8 and >256 μ g/ml, respectively, the ratio was scored as $512/8 = 64$.

^b PIPTAZ, piperacillin-tazobactam.

^c Comprising 4 *C. freundii* isolates, 3 *E. cloacae* isolates, 5 *K. pneumoniae* isolates, 5 *M. morgani* isolates, and 2 *S. marcescens* isolates.

^d Comprising 3 *C. freundii* isolates, 2 *E. cloacae* isolates, 2 *M. morgani* isolates, and 2 *S. marcescens* isolates.

DISCUSSION

Established carbapenems have a deserved reputation for activity against ESBL-producing and AmpC-derepressed *Enterobacteriaceae*, with resistance arising only when these mechanisms are combined with impermeability (5, 16, 17). We examined whether ertapenem shared this favorable behavior and tested its activity against strains with molecular class A and B carbapenemases.

Ertapenem was strongly active against ESBL-producing *Klebsiella* isolates and AmpC-derepressed *Enterobacteriaceae*, with MICs mostly ca. 0.03 to 0.12 μ g/ml and always ≤ 4 μ g/ml. Nevertheless, ertapenem was ca. fourfold less active against ESBL producers than against nonproducers (Table 1); more-

over, *E. cloacae* and *C. freundii* strains that hyperproduced AmpC enzymes were less susceptible than their AmpC-basal mutants (Table 2). These data suggest that ertapenem is slightly less stable in the presence of β -lactamase than imipenem and meropenem, which retain full activity against ESBL producers and against AmpC-hyperproducing *E. cloacae* and *C. freundii* (2, 3, 16, 25). To investigate this aspect, ESBL-producing and AmpC-derepressed strains were examined for inoculum effects. In general, the effects of β -lactamase lability on MICs become more apparent as the inoculum is raised, as seen here with cefepime against ESBL producers and with piperacillin against control klebsiellas. The inoculum effects observed with ertapenem were small, less even than with imipenem, and substantial lability in the presence of ESBLs and

TABLE 4. MICs of ertapenem and drugs compared for carbapenemase-producing *Enterobacteriaceae*

Strain and enzyme ^a	MIC (μ g/ml) of:					
	Ertapenem	Imipenem	Ceftazidime	Cefepime	Piperacillin	PIPTAZ ^b
<i>K. pneumoniae</i> K4181 IMP-1 ⁺ 39-kDa OMP ⁻	>32	>32	>128	>128	>256	>256
<i>K. pneumoniae</i> K4181 IMP-1 ⁺ 39-kDa OMP ⁺	6	2	>128	>128	>256	>256
<i>S. marcescens</i> S6 SME-1	2	>32	0.25	0.5	8	4

^a OMP, outer membrane protein.

^b PIPTAZ, piperacillin-tazobactam.

AmpC enzymes could therefore be discounted. The slightly increased MICs of ertapenem for some ESBL producers and AmpC-derepressed strains may reflect other factors. We note by analogy that the MICs of cefoxitin for many ESBL-positive klebsiellas exceed those for ESBL-negative strains (2, 19) but that ESBL-coding plasmids do not raise the MICs of cefoxitin for *E. coli* transconjugants (13).

AmpC-inducible *E. cloacae* and *C. freundii* were more susceptible than their derepressed mutants to ertapenem but less susceptible than the corresponding AmpC- basal mutants. This observation implies that ertapenem, like biapenem and sanfetrinem (3, 6) but unlike imipenem (25), is not a strong inducer of AmpC enzymes in MIC tests. Confirmation of this inference must, however, await direct induction assays.

Acquired carbapenemases are rare but are increasingly being reported, mostly from nonfermenters but occasionally from *Enterobacteriaceae*. Interest has centered on the IMP and VIM metallo- β -lactamases, which belong to molecular class B, but carbapenemase activity has also been found in a few class A and D enzymes. IMP enzymes are scattered in *Pseudomonas aeruginosa* and *Serratia* in Japan and have been found also in isolates from Canada, China, Italy, Hong Kong, and Singapore, (7, 12, 18, 24); VIM enzymes have been found in *P. aeruginosa* isolates from widely scattered sites across Eurasia (18). Class D β -lactamases with carbapenemase activity have been found in *Acinetobacter* spp. worldwide but not in other genera (1, 4, 8); class A β -lactamases able to hydrolyze carbapenems have been found in tiny numbers of *Serratia* and *Enterobacter* spp. from Europe and North America (18). *K. pneumoniae* K4181 with IMP-1 β -lactamase and lacking a 39-kDa porin was resistant to all β -lactams tested, including ertapenem. Resistance to carbapenems, but not to other antibiotics, was reduced when expression of the porin was restored. The view that IMP enzymes require impermeability (or some other factor) to confer carbapenem resistance is supported by the observations that imipenem MICs for *E. coli* transconjugants with the IMP-1 enzyme are only ca. 2 μ g/ml (14, 23) and that many *bla*_{IMP}⁺*P. aeruginosa* isolates express resistance to ceftazidime but not to imipenem (24). *S. marcescens* S6, with the SME-1 enzyme (21), was resistant to imipenem and had reduced susceptibility to ertapenem (MIC, 2 μ g/ml) as well as to meropenem (MIC, 2 μ g/ml [3, 21]). It is unclear whether these MICs equate to clinical resistance; they are ca. 32-fold higher than those for typical *S. marcescens* strains but still below the NCCLS breakpoints for imipenem and meropenem, which are as follows: susceptible, ≤ 4 μ g/ml; resistant, ≥ 16 μ g/ml (22). Provisional NCCLS MIC breakpoints for ertapenem are identical (NCCLS summary minutes of the meeting of the Subcommittee on Antimicrobial Susceptibility Testing, 7 to 9 June 1998, p. 15–16) but await formal confirmation and ratification.

In summary, ertapenem has acceptable stability in the presence of AmpC and ESBLs, which are increasingly widespread in current isolates. In the future, ertapenem and other carbapenems may be threatened by the spread of IMP-1 and other metallo- β -lactamases. The magnitude of this threat is likely to depend on the level of usage, and suitable surveillance in warranted. Spread of SME-1-like enzymes seems a lesser hazard, as none has ever been shown to be transferable among bacteria.

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REFERENCES

1. Afzal-Shah, M., N. Woodford, and D. M. Livermore. 2001. Characterization of OXA-25, -26, and -27: molecular class D β -lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **45**:583–588.
2. Babini, G. S., and D. M. Livermore. 2000. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997–1998. *J. Antimicrob. Chemother.* **45**:183–189.
3. Babini, G. S., M. Yuan, and D. M. Livermore. 1998. Interactions of β -lactamases with sanfetrinem (GV 104326) compared to those with imipenem and with oral β -lactams. *Antimicrob. Agents Chemother.* **42**:1168–1175.
4. Bou, G., A. Oliver, and J. Martinez-Beltran. 2000. OXA-24: a novel class D β -lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. *Antimicrob. Agents Chemother.* **44**:1556–1561.
5. Bradford, P. A., C. Urban, N. Mariano, S. J. Projan, J. J. Rahal, and K. Bush. 1997. 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.
6. Chen, H. Y., and D. M. Livermore. 1994. In-vitro activity of biapenem, compared with imipenem and meropenem, against *Pseudomonas aeruginosa* strains and mutants with known resistance mechanisms. *J. Antimicrob. Chemother.* **33**:949–958.
7. Cornaglia, G., M. L. Riccio, A. Mazzariol, L. Lauretti, R. Fontana, and G. M. Rossolini. 1999. Appearance of IMP-1 metallo- β -lactamase in Europe. *Lancet* **353**:899–900.
8. Donald, H. M., W. Scaife, S. G. Amies, and H. K. Young. 2000. Sequence analysis of ARI-1, a novel OXA β -lactamase responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. *Antimicrob. Agents Chemother.* **44**:196–199.
9. Edwards, J. R., and M. J. Betts. 2000. Carbapenems: the pinnacle of the β -lactam antibiotics or room for improvement? *J. Antimicrob. Chemother.* **45**:1–4.
10. Essack, S. Y., L. M. C. Hall, D. G. Pillay, M. L. McFadyen, and D. M. Livermore. 2001. Complexity and diversity of *Klebsiella pneumoniae* strains with extended-spectrum β -lactamases in 1994 and 1996 at a teaching hospital in Durban, South Africa. *Antimicrob. Agents Chemother.* **45**:88–95.
11. Gheorghiu, R., M. Yuan, L. M. Hall, and D. M. Livermore. 1997. Bases of variation in resistance to β -lactams in *Klebsiella oxytoca* isolates hyperproducing K1 β -lactamase. *J. Antimicrob. Chemother.* **40**:533–541.
12. Iyobe, S., H. Kusadokoro, J. Ozaki, N. Matsumura, S. Minami, S. Haruta, T. Sawai, and K. O'Hara. 2000. Amino acid substitutions in a variant of IMP-1 metallo- β -lactamase. *Antimicrob. Agents Chemother.* **44**:2023–2027.
13. Jacoby, G. A., and I. Carreras. 1990. Activities of β -lactam antibiotics against *Escherichia coli* strains producing extended-spectrum β -lactamases. *Antimicrob. Agents Chemother.* **34**:858–862.
14. Koh, T. H., G. S. Babini, N. Woodford, L. H. Sng, L. M. Hall, and D. M. Livermore. 1999. Carbapenem-hydrolysing IMP-1 β -lactamase in *Klebsiella pneumoniae* from Singapore. *Lancet* **353**:2162.
15. Kohler, J., K. L. Dorso, K. Young, G. G. Hammond, H. Rosen, H. Kropp, and L. L. Silver. 1999. In vitro activities of the potent, broad-spectrum carbapenem MK-0826 (L-749345) against broad-spectrum β -lactamase- and extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates. *Antimicrob. Agents Chemother.* **43**:1170–1176.
16. Livermore, D. M. 1995. β -Lactamases in laboratory and clinical resistance. *Clin. Microbiol. Rev.* **8**:557–584.
17. Livermore, D. M. 1997. Acquired carbapenemases. *J. Antimicrob. Chemother.* **39**:673–676.
18. Livermore, D. M., and N. Woodford. 2000. Carbapenemases: a problem in waiting? *Curr. Opin. Microbiol.* **3**:489–495.
19. Livermore, D. M., and M. Yuan. 1996. Antibiotic resistance and production of extended-spectrum β -lactamases amongst *Klebsiella* spp. from intensive care units in Europe. *J. Antimicrob. Chemother.* **38**:409–424.
20. M'Zali, F. H., J. Heritage, D. M. Gascogne-Binzi, M. Denton, N. J. Todd, and P. M. Hawkey. 1997. Transcontinental importation into the United Kingdom of *Escherichia coli* expressing a plasmid-mediated AmpC-type β -lactamase exposed during an outbreak of SHV-5 extended-spectrum β -lactamase in a Leeds hospital. *J. Antimicrob. Chemother.* **40**:823–831.
21. Naas, T., L. Vandel, W. Sougakoff, D. M. Livermore, and P. Nordmann. 1994. Cloning and sequence analysis of the gene for a carbapenem-hydrolysing class A β -lactamase, Sme-1, from *Serratia marcescens* S6. *Antimicrob. Agents Chemother.* **38**:1262–1270.
22. National Committee for Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7–A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
23. Osano, E., Y. Arakawa, R. Wacharotayankun, M. Ohta, T. Horii, H. Ito, F.

- Yoshimura, and N. Kato. 1994. Molecular characterization of an enterobacterial metallo- β -lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. *Antimicrob. Agents Chemother.* **38**:71–78.
24. Senda, K., Y. Arakawa, K. Nakashima, H. Ito, S. Ichiyama, K. Shimokata, N. Kato, and M. Ohta. 1996. Multifocal outbreaks of metallo- β -lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum β -lactams, including carbapenems. *Antimicrob. Agents Chemother.* **40**:349–353.
25. Yang, Y. J., and D. M. Livermore. 1989. Interactions of meropenem with class I chromosomal β -lactamases. *J. Antimicrob. Chemother.* **24**(Suppl. A): 207–217.
26. Yuan, M. 2000. PhD thesis. University of London, London, United Kingdom.
27. Yuan, M., H. Aucken, L. M. Hall, T. L. Pitt, and D. M. Livermore. 1998. Epidemiological typing of klebsiellae with extended-spectrum β -lactamases from European intensive care units. *J. Antimicrob. Chemother.* **41**:527–539.