

## **Antibacterial properties of imipenem with special reference to the activity against methicillin-resistant staphylococci, cefotaxime-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa***

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Imipenem was examined with standardized agar dilution procedures against a wide range of bacteria. Geometric mean MICs against the genera *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Serratia* were 0.1–0.4 mg/l, and *Proteus* and *Providencia* spp. were inhibited by 0.25–4 mg/l. *Acinetobacter calcoaceticus* var. *anitratum* strains were inhibited by concentrations ranging from 0.12–0.5 mg/l. Methicillin-susceptible staphylococci were highly susceptible to the drug (MICs:  $\leq 0.03$  mg/l) and enterococci were inhibited by 0.25–16 mg/l. Most of the multi-resistant JK corynebacteria were resistant to imipenem. Imipenem was more active than any other  $\beta$ -lactam against methicillin-resistant staphylococci; this was also demonstrated in a population analysis. Imipenem-resistant minorities in populations, however, were also observed. Cefotaxime-resistant and -intermediate *Enterobacter* and *Citrobacter* strains were inhibited by concentrations of 0.5 mg/l or less. No third-generation cephalosporin nor any other  $\beta$ -lactam showed similarly high activity against these groups of organisms. Among 20 ceftazidime-resistant and 20 ceftazidime-susceptible isolates of *Pseudomonas aeruginosa*, no strain was resistant and only five ceftazidime-resistant strains were intermediately susceptible (MIC, 8 mg/l) to imipenem.

### **Introduction**

The aim of this work was to study the in-vitro activity of imipenem in comparison with other broad-spectrum  $\beta$ -lactam antibiotics against a wide range of bacteria.

### **Materials and methods**

#### *Organisms*

The bacterial strains used were recently isolated from clinical specimens and identified according to standard procedures.

#### *Antibiotics*

Imipenem (Merck, Sharp & Dohme), ceftazidime (Glaxo), aztreonam (Squibb), cefotaxime (Hoechst), cefpirome (HR810) (Hoechst-Roussel), ceftriaxone (Hoffman La Roche), cefoperazone (Pfizer) and latamoxef (Lilly) were kindly supplied by the manufacturers.

**Table I.** In-vitro activity of imipenem and seven  $\beta$ -lactam antibiotics against Enterobacteriaceae

Organisms (no. of strains)	Drug	MIC (mg/l)			Geometric mean
		50% <sup>a</sup>	90% <sup>a</sup>	Range	
<i>Esch. coli</i> (12)	imipenem	0.12	0.25	≤0.03–0.5	0.11
	ceftazidime	8	16	0.12–16	2.1
	aztreonam	4	8	0.06–8	1.1
	cefpime	0.06	0.25	≤0.03–1	0.09
	cefotaxime	2	4	0.06–4	0.89
	ceftriaxone	1	1	≤0.03–1	0.40
	cefoperazone	2	16	0.5–64	2.0
	latamoxef	0.5	1	0.06–1	0.33
<i>Klebsiella</i> spp. (27)	imipenem	0.12	0.25	0.06–1	0.15
	ceftazidime	0.25	0.5	0.06–16	0.23
	aztreonam	8	32	≤0.03–64	1.8
	cefpime	0.5	2	≤0.03–4	0.36
	cefotaxime	0.5	2	0.06–16	0.38
	ceftriaxone	2	64	≤0.03–128	1.1
	cefoperazone	128	256	0.25–512	38.3
	latamoxef	0.12	1	≤0.03–16	0.14
<i>Enterobacter</i> spp. cefotaxime susceptible (11)	imipenem	0.25	1	0.06–1	0.30
	ceftazidime	1	16	0.25–16	1.6
	aztreonam	0.25	4	≤0.03–16	0.47
	cefpime	0.25	0.5	≤0.03–0.5	0.22
	cefotaxime	2	8	0.06–8	1.4
	ceftriaxone	4	8	0.06–16	1.5
	cefoperazone	8	8	0.06–16	3.3
	latamoxef	0.5	1	0.06–1	0.34
<i>Enterobacter</i> spp. cefotaxime intermediate/resistant (21)	imipenem	0.25	0.5	≤0.03–0.5	0.20
	ceftazidime	128	256	16–>512	101.6
	aztreonam	32	64	4–128	32.0
	cefpime	4	8	0.5–128	2.6
	cefotaxime	128	256	16–>512	115.9
	ceftriaxone	128	512	16–>512	119.8
	cefoperazone	64	256	16–>512	89.0
	latamoxef	8	16	0.5–128	6.1
<i>C. freundii</i> cefotaxime susceptible (13)	imipenem	0.25	0.25	0.12–1	0.24
	ceftazidime	0.25	4	0.12–4	0.62
	aztreonam	0.25	2	≤0.03–2	0.29
	cefpime	0.12	0.5	≤0.03–1	0.14
	cefotaxime	1	4	0.06–4	0.85
	ceftriaxone	0.5	8	≤0.03–8	0.56
	cefoperazone	8	16	0.12–64	5.5
	latamoxef	0.06	0.5	0.06–8	0.16
<i>C. freundii</i> cefotaxime intermediate/resistant (17)	imipenem	0.12	0.25	0.12–0.25	0.15
	ceftazidime	128	512	32–512	156.9
	aztreonam	32	128	8–256	39.2
	cefpime	2	4	0.25–4	1.6
	cefotaxime	64	128	16–128	50.1
	ceftriaxone	64	256	16–256	81.7
	cefoperazone	64	256	32–256	81.7
	latamoxef	2	8	1–32	3.7

Table I—*contd.*

Organisms (no. of strains)	Drug	MIC (mg/l)			Geometric mean
		50% <sup>a</sup>	90% <sup>a</sup>	Range	
<i>Ser. marcescens</i> (25)	imipenem	0.5	0.5	0.12–1	0.41
	ceftazidime	0.5	2	0.06–16	0.44
	aztreonam	0.5	8	0.06–8	0.74
	cefpirome	0.5	1	≤0.03–4	0.34
	cefotaxime	8	32	0.12–32	3.1
	ceftriaxone	2	16	0.06–32	1.7
	cefoperazone	64	128	8–128	43.4
	latamoxef	0.25	8	0.12–16	0.76
<i>Proteus</i> and <i>Providencia</i> spp. <sup>b</sup> (19)	imipenem	1	2	0.25–4	0.75
	ceftazidime	0.06	0.5	≤0.03–1	0.05
	aztreonam	≤0.03	0.03	≤0.03–0.06	≤0.03
	cefpirome	0.5	4	0.12–4	0.77
	cefotaxime	≤0.03	0.06	≤0.03–0.25	≤0.03
	ceftriaxone	≤0.03	0.03	≤0.03–0.06	≤0.03
	cefoperazone	≤0.03	0.03	≤0.03–0.06	≤0.03
	latamoxef	0.25	0.5	≤0.03–0.5	0.17

<sup>a</sup>MIC<sub>50</sub> and MIC<sub>90</sub> are the concentrations of antibiotics required to inhibit 50% and 90% of the examined strains, respectively.

<sup>b</sup>*Prot. mirabilis* (6), *Prot. vulgaris* (6), *Prov. rettgeri* (4), *Prov. stuartii* (3).

## Methods

Minimal inhibitory concentrations (MICs) were determined according to the NCCLS agar-dilution procedure (National Committee for Clinical Laboratory Standards (NCCLS) (1985)). Mueller–Hinton medium with 3% agar content was used to prevent swarming of *Providencia* and *Proteus* spp. Gonococci (GC) agar base supplemented with 5% defibrinated sheep blood was used for JK corynebacteria.

A population of methicillin-resistant staphylococci was analysed by disaggregation of overnight broth cultures (brief ultrasonication at 20 kHz) and surface inoculation on to drug-containing Mueller–Hinton agar plates with 0.1 ml of appropriate dilutions of the processed broth. Incubation was at 37°C for 48 h. Colony counts served to calculate the number of resistant cells in a population of 10<sup>8</sup> cfu.

## Results

The MICs for the Gram-negative and Gram-positive organisms used to define the spectrum of imipenem are presented in Tables I to III. Imipenem was highly active against the Enterobacteriaceae; exceeding the activity of most of the other  $\beta$ -lactam antibiotics tested (Table I). The drug showed less activity against *Proteus* and *Providencia* spp., but MICs were still below the susceptibility threshold of ≤4 mg/l. Imipenem was equally active against *Enterobacter* and *Citrobacter* strains resistant or susceptible to cefotaxime. Most of the cefotaxime-resistant strains were also resistant to ceftazidime, aztreonam, ceftriaxone and cefoperazone. Cefpirome and latamoxef

**Table II.** In-vitro activity of imipenem and seven  $\beta$ -lactam antibiotics against *A. anitratus* and *Ps. aeruginosa*

Organisms (no. of strains)	Drug	MIC (mg/l)			Geometric mean
		50%	90%	Range	
<i>A. calcoaceticus</i> var. <i>anitratum</i> (11)	imipenem	0.25	0.5	0.12-0.5	0.21
	ceftazidime	8	16	4-16	8.5
	aztreonam	32	64	16-64	36.3
	ceftiofime	32	64	1-64	20.6
	cefotaxime	16	32	4-32	17.0
	ceftriaxone	16	32	4-32	14.1
	cefoperazone	128	256	128-256	164.7
	latamoxef	16	16	2-32	13.2
<i>Ps. aeruginosa</i> ceftazidime susceptible (20)	imipenem	1	2	0.5-4	1.1
	ceftazidime	2	4	1-8	2.1
	aztreonam	4	16	2-32	5.9
	ceftiofime	8	16	2-64	7.7
	cefotaxime	16	64	8-256	26.0
	ceftriaxone	32	128	8-256	29.9
	cefoperazone	8	64	4-128	11.3
	latamoxef	16	32	8-128	16.0
<i>Ps. aeruginosa</i> ceftazidime intermediate/resistant (20)	imipenem	4	8	0.5-8	2.8
	ceftazidime	64	64	16-256	46.9
	aztreonam	64	128	8-128	50.2
	ceftiofime	256	512	2-512	119.4
	cefotaxime	512	< 512	32-> 512	512
	ceftriaxone	> 512	> 512	32-> 512	675.6
	cefoperazone	256	512	8-> 512	230.7
	latamoxef	128	256	16-512	152.2

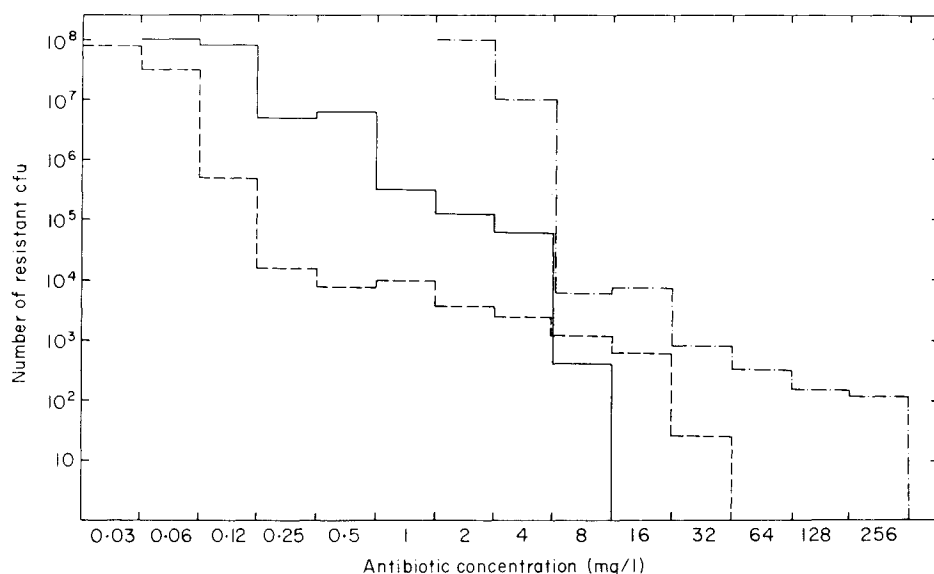
exerted good activity against these problem strains, although the geometric means of their MICs were 10-20 times higher than against cefotaxime-susceptible *Enterobacter* and *Citrobacter* spp.

Imipenem was the most active amongst the agents tested against *Acinetobacter calcoaceticus* var. *anitratum* and *Pseudomonas aeruginosa*, whether susceptible or resistant to ceftazidime (Table II). MICs of imipenem against ceftazidime-resistant *Ps. aeruginosa* were only slightly higher than against susceptible strains. This finding was in sharp contrast to the results obtained with the other  $\beta$ -lactams. The latter all showed highly elevated MICs against the ceftazidime-resistant strains. Cross-resistance was not observed between imipenem and the other drugs examined. Imipenem was highly active against methicillin-susceptible staphylococci (Table III). Compared with the activity against other Gram-positive bacteria (Tischhauser & Kayser, 1983), the drug's activity against enterococci was relatively low. However, 50% of the enterococci were inhibited by 1 mg/l and 90% by 8 mg/l of the drug, respectively. These values were much lower than those of the other  $\beta$ -lactams examined. Antistaphylococcal activity of imipenem was generally superior to that of the other  $\beta$ -lactams tested. Methicillin-resistant staphylococci were inhibited by higher imipenem concentrations than susceptible isolates but only 5% of the strains had MICs above the susceptibility

**Table III.** In-vitro activity of imipenem and six  $\beta$ -lactam antibiotics against Gram-positive organisms

Organisms (no. of strains)	Drug	MIC (mg/l)			Geometric mean
		50%	90%	Range	
<i>Staph. aureus</i> methicillin susceptible (19)	imipenem	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$
	ceftazidime	8	16	4-16	8.9
	cefpime	1	1	0.12-1	0.58
	cefotaxime	2	4	0.5-4	1.7
	ceftriaxone	4	8	1-8	3.0
	cefoperazone	2	4	1-4	1.9
	latamoxef	16	16	8-16	9.3
<i>Staph. aureus</i> methicillin resistant (19)	imipenem	$\leq 0.03$	0.12	$\leq 0.03$ -64	0.07
	ceftazidime	32	128	4-512	32.0
	cefpime	2	32	0.5-64	2.5
	cefotaxime	16	128	1->512	12.4
	ceftriaxone	16	256	2->512	17.9
	cefoperazone	16	32	1->512	15.4
	latamoxef	32	128	8->512	33.2
Coagulase-negative staphylococcus methicillin susceptible (16)	imipenem	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$
	ceftazidime	8	16	4-16	6.7
	cefpime	0.25	0.5	0.12-2	0.25
	cefotaxime	1	4	0.5-8	1.6
	ceftriaxone	2	4	0.5-8	1.5
	cefoperazone	2	4	0.5-4	1.5
	latamoxef	16	32	8-32	18.2
Coagulase-negative staphylococcus methicillin resistant (20)	imipenem	0.06	0.5	$\leq 0.03$ -16	0.09
	ceftazidime	8	32	0.5-32	8.9
	cefpime	0.5	4	0.06-16	0.71
	cefotaxime	2	8	0.06-16	2.3
	ceftriaxone	4	32	0.12-32	4.4
	cefoperazone	2	4	0.25-8	2.1
	latamoxef	32	64	8-128	34.3
Enterococci (20)	imipenem	1	8	0.25-16	1.9
	ceftazidime	> 512	> 512	128->512	749.6
	cefpime	16	512	1->512	34.3
	cefotaxime	256	512	32->512	294.1
	ceftriaxone	128	512	4->512	123.6
	cefoperazone	64	512	8-512	64.0
	latamoxef	> 512	> 512	128->512	861.1
Multiresistant JK corynebacteria (14)	imipenem	256	512	0.5->512	115.9
	ceftazidime	> 512	> 512	128->512	655.8
	cefpime	512	> 512	4->512	141.3
	cefotaxime	> 512	> 512	8->512	269.0
	ceftriaxone	> 512	> 512	8->512	243.6
	cefoperazone	> 512	> 512	16->512	231.9
	latamoxef	> 512	> 512	256->512	760.8

breakpoint of 4 mg/l. Figure 1 shows the population analysis of the penicillinase-negative *Staphylococcus aureus* strain FK 362 with respect to imipenem, penicillin G and methicillin. Although heterogeneity was also found with imipenem, the population was more susceptible to imipenem than to penicillin G and to methicillin.



**Figure 1.** Number of viable units resistant to each concentration of antimicrobial agent remaining after plating an inoculum of  $10^8$  cfu of methicillin-resistant,  $\beta$ -lactamase-negative *Staph. aureus* strain FK 362 and incubating at  $37^\circ\text{C}$  for 48 h (--- imipenem; — penicillin G; —·— methicillin).

### Discussion

An outstanding property of imipenem is its activity against strains of Enterobacteriaceae resistant to third-generation cephalosporins. This property may be due to the stability of imipenem to the chromosomal cephalosporinase responsible for resistance in these organisms (Livermore, Williams & Williams, 1981; Seeberg, Tolxdorff-Neutzing & Wiedemann, 1983) as well as its high penetrability through the outer membrane of Gram-negative bacteria due to its zwitterionic character and compact molecular structure (Yoshimura & Nikaido, 1985). Stability to  $\beta$ -lactamases and good penetrability of the cell wall also could explain the activity of imipenem against ceftazidime-resistant *Ps. aeruginosa*.

Another outstanding feature of imipenem is its activity against Gram-positive cocci including the enterococci. The majority of the methicillin-resistant staphylococci were susceptible to imipenem in standard agar dilution tests, but with an inoculum of  $10^4$  cells per spot and incubation for 20 h at  $35^\circ\text{C}$ , these tests were unfavourable for the expression of intrinsic resistance to  $\beta$ -lactam antibiotics in staphylococci. The majority of cells in a population of methicillin-resistant *Staph. aureus* will be susceptible to imipenem, but minorities of a few more or less resistant cells may occur. Despite this situation, imipenem seems to be an effective agent in the treatment of infections caused by methicillin-resistant staphylococci (Fan *et al.*, 1986).

Thus, imipenem presents an impressive potential in the therapy of bacterial infections.

### References

- Fan, W., Del Busto, R., Love, M., *et al.* (1986). Imipenem-cilastatin in the treatment of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* infections. *Antimicrobial Agents and Chemotherapy* **29**, 26–9.

- Livermore, D. M., Williams, R. J. & Williams, J.D. (1981). In-vitro activity of MK 0787 (*N*-formimidoylthienamycin) against *Pseudomonas aeruginosa* and other Gram-negative organisms and its stability to their  $\beta$ -lactamases. *Journal of Antimicrobial Chemotherapy* **8**, 355–62.
- National Committee for Clinical Laboratory Standards (1985). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard NCCLS publication M7-A. NCCLS, Villanova, Pa.
- Seeberg, A. H., Tolxdorff-Neutzling, R. M. & Wiedemann, B. (1983). Chromosomal beta-lactamases of *Enterobacter cloacae* are responsible for resistance to third-generation cephalosporins. *Antimicrobial Agents and Chemotherapy* **23**, 918–25.
- Tischhauser, G. & Kayser, F. H. (1983). The in vitro activity of *N*-formimidoyl thienamycin compared with other broad-spectrum cephalosporins and with clindamycin and metronidazole. *Infection* **11**, 219–26.
- Yoshimura, F. & Nikaïdo, H. (1985). Diffusion of beta-lactam antibiotics through the porin channels of *Escherichia coli* K12. *Antimicrobial Agents and Chemotherapy* **27**, 84–92.