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Bioactivity of Imipenem as a Function of Medium, Time, and Temperature

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The bioactivity of imipenem at 20 μg/ml in various agar and broth media which are commonly used in susceptibility test assays was measured at different storage temperatures over time. Imipenem was found to be more stable at 4°C than at −20°C and least stable in all media at 35°C.

Imipenem, a beta-lactam antimicrobial agent, has been reported to demonstrate activity against a variety of microorganisms (1, 3). The stability of this agent incorporated into cation-supplemented Mueller-Hinton broth (CSMHB) in microdilution trays is in excess of 1 year when frozen at continuous temperatures of −70°C or lower (MK-0787 susceptibility powder package insert; Merck Sharp & Dohme Research Laboratories, Rahway, N.J.); the bioactivity of imipenem in broth media at other temperatures or in agar media has not been reported. We used a modification of the agar diffusion assay (4) to measure the bioactivity of imipenem in Middlebrook 7H-11 agar, Wilkins-Chalgren agar, and Mueller-Hinton agar at three temperatures. We also assayed the bioactivity of imipenem in CSMHB, CSMHB with 5% Fildes enrichment, Todd-Hewitt broth, and brain heart infusion broth at 35°C, and in CSMHB at the additional temperatures of −20°C in a non-frost-free freezer, 4°C, and 25°C.

(This work was presented in part at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, Nev. [E. J. Baron and J. A. Hindler, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 553, 1983].)

Microbiological assay seed agar plates were prepared in a single batch as follows: *Micrococcus lysodeikticus* (UCLA stock culture, maintained at −70°C) was inoculated into brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and incubated at 36°C until the culture reached the late-logarithmic phase. The suspension was adjusted to match the turbidity of a McFarland 0.5 standard in sterile distilled water, and 1.5 ml was added to a tube of 18 ml of melted 1% modified Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) for each plate prepared. The seeded agar was poured into a polystyrene petri plate (15 by 150 mm) on a level surface, and the agar was allowed to solidify. Those plates not used immediately were sealed in plastic bags and refrigerated. The seed agar plates remained stable for a minimum of 24 days at 4°C (data not shown).

Imipenem reconstituted in 0.1 M phosphate buffer (pH 7.0) from laboratory reference standard powder (kindly provided by H. Kropp, Merck Institute for Therapeutic Research) to a final concentration of 20 μg/ml was incorporated into the four broth media to be tested, all purchased from Difco Laboratories. These imipenem-containing broth media were dispensed in 1.0-ml volumes into polystyrene tubes (12 by 75 mm; BD Labware, Oxnard, Calif.) and stored at the temperatures to be tested. Test solutions held at −20°C were thawed at the time of bioassay and were not refrozen.

Imipenem (20 μg/ml) was added to 20-ml volumes of melted 50°C Wilkins-Chalgren, Middlebrook 7H-11, and Mueller-Hinton agars, also purchased from Difco Laboratories. The agar solutions were poured into individual plastic petri dishes (diameter, 100 mm). After solidification, the agar plates were stored in tightly sealed plastic bags at each of the respective temperatures studied.

Bioactivity was determined by measuring the zone of inhibition of growth of *M. lysodeikticus* around either a well in the seed agar containing 5.5 μl of imipenem-containing broth medium or a plug of imipenem-containing agar medium applied to the surface of the seed agar. In either case, the wells or plugs were produced with a single no. 1 size cork borer, with a 3-mm external diameter. Seed agar plates were refrigerated (4°C) for 1 h after application of the test media to allow prediffusion of the imipenem and then incubated for 24 h at 36°C in air. Zone diameters were measured with a Transidyne calibrating viewer (Transidyne General Corp.,

TABLE 1. Percentage of initial concentration of imipenem in agars and broth stored at different temperatures

Day	% Initial concn of imipenem in:												
	Agar									CSMHB			
	Middlebrook 7H-11			Wilkins-Chalgren			Mueller-Hinton						
	35°C	25°C	4°C	35°C	25°C	4°C	35°C	25°C	4°C	35°C	25°C	4°C	−20°C
0	100	100	100	100	100	100	100	100	100	100	100	100	100
1	33	78		63	90		60	85		70	94		
2	0	45	100	27	84	100	6	73	100	10	92	100	100
3		13		0	62		0	60		0	65		
4		0	94		60	100		35	100		40	100	70
5					48			17			22		
6			92		41	100		6	100		0	84	60
7					37			0					
8			80		18	100			100			83	40
9					10								
10			69		0	100			100			82	18
12			54									55	9
14			40			100			100			50	0
16			23									48	
18			4									44	
20			0			100			100			40	
22												20	
24						100			100			0	

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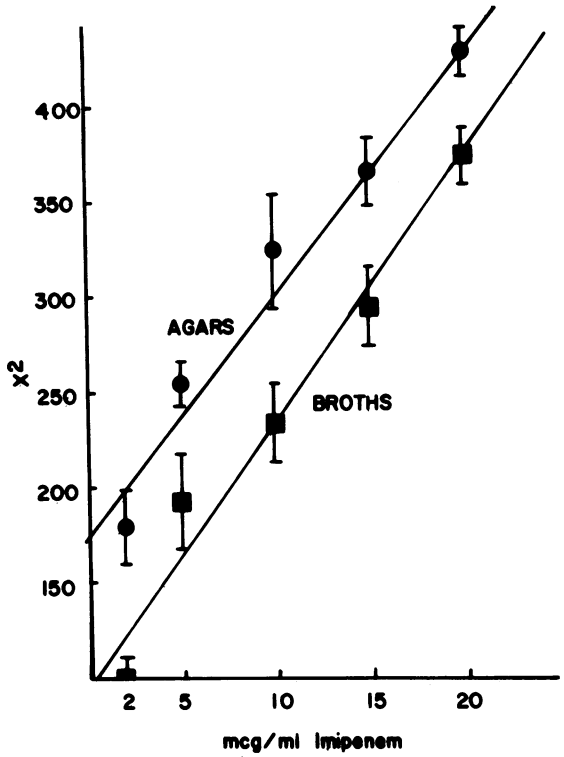

FIG. 1. Standard curve comparing concentration of imipenem to zone of inhibition of *M. lysodeikticus*. The values for X^2 are derived from the zone diameter (d) by the formula: $X = 0.5\ (d - 3)$, in which 3 mm was the diameter of the wells or plugs. Each point represents the mean and standard deviation of five separate determinations. Lines were derived by linear regression analysis. mcg, Micrograms.

Ann Arbor, Mich.). All determinations were performed double blinded and in duplicate on separate bioassay agar plates (four measurements per point). Zone diameters were compared to a standard curve of imipenem concentration versus diameter derived by using the Cooper equation and methods delineated by Kavanagh (2) (Fig. 1).

Bioactivity of imipenem declined over time in Todd-Hewitt and brain heart infusion broth media even more rapidly than in CSMHB incubated at 35°C (data not shown). Bioactivity of imipenem in CSMHB was substantially reduced after 1 day of incubation (Table 1). Loss of bioactivity of imipenem in CSMHB occurred faster at −20°C than at 4°C. In contrast, no decrease in bioactivity occurred over extended periods at −70°C in our laboratory (data not shown) or during studies of others (MK-0787 susceptibility powder package insert; Merck Sharp & Dohme Research Laboratories; D. J. Nickolai, C. J. Lammel, B. A. Byford, J. H. Morris, E. B. Kaplan, K. W. Hadley, and G. F. Brooks, Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 552, 1983). Differential freezing of the water portion of the solution at −20°C, allowing the reactants to interact in a more concentrated milieu which is not solidly frozen at this temperature, may contribute to this phenomenon. Of the solid agar media, imipenem incorporated into Middlebrook 7H-11 agar was least stable at all temperatures tested (Table 1). At 4°C, imipenem retained 100% bioactivity for at least 3 weeks in Mueller-Hinton and Wilkins-Chalgren agars (Table 1).

These results imply that performing susceptibility tests on organisms that require prolonged incubation at 35°C will present difficulties. We have developed an agar dilution susceptibility test procedure which counteracts some of these problems (E. J. Baron, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C302, p. 287). The problems that the lability of imipenem and other beta-lactam antimicrobics (23rd ICAAC, abstr. no. 552) presents for microbroth susceptibility testing systems are more immediate, however. The storage of imipenem-containing susceptibility testing broth media at −20°C must be limited, which compromises its usefulness in some of the commercially prepared microdilution panels, which are currently intended to be stored at −20°C.

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