

## Comparative Activities of Piperacillin and Tazobactam against Clinical Isolates of *Legionella* spp.

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**We evaluated the in vitro activity of piperacillin alone or in combination with the  $\beta$ -lactamase inhibitor tazobactam against clinical isolates of *Legionella* species. At an inoculum of approximately  $10^4$  CFU, tazobactam, piperacillin, and the 8:1 combination had equivalent activities against *Legionella* spp. At an approximately 10-fold higher inoculum, the following results were obtained, expressed as MICs for 50 and 90% of strains tested (MIC range): piperacillin, 4 and 16 (0.25 to 32)  $\mu$ g/ml; tazobactam, 0.5 and 1 (0.125 to 2)  $\mu$ g/ml; and piperacillin-tazobactam (expressed in terms of MIC of piperacillin) 0.5 and 1 (0.03 to 2)  $\mu$ g/ml. Tazobactam alone and the combination with piperacillin were more active than piperacillin alone at the higher inoculum.**

$\beta$ -Lactams are widely used in the treatment of bacterial pneumonias. However, when other bacterial pathogens are not readily identifiable, concern for a possible etiologic role of *Legionella* species often results in the addition of erythromycin to  $\beta$ -lactams in empirically selected antimicrobial regimens. Several  $\beta$ -lactam antibiotics actually demonstrate activity against *Legionella* spp. in vitro on various media (9, 11), but a number of these are ineffective when examined against intracellular *Legionella* strains in vitro or in animal models of infection with these organisms (3, 13, 14, 16). A notable exception to these observations is the fact that both clavulanic acid alone and combinations of this agent with either amoxicillin or ticarcillin have demonstrated activity against cell-associated *Legionella pneumophila* in vitro and in experimental animal models (13, 14, 16).

In the present study we evaluated the in vitro activities of piperacillin and the  $\beta$ -lactamase inhibitor tazobactam, alone and in fixed 8:1 combination, against *Legionella* spp. We examined the influence of inoculum size on susceptibility to the antibiotics and compared these agents with sulbactam, clavulanic acid, ampicillin, cefuroxime, cefotaxime, erythromycin, clarithromycin, ciprofloxacin, and rifampin under the same experimental conditions.

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The 29 unique clinical *Legionella* isolates tested included *L. pneumophila* (16 strains), *L. longbeachae* (3 strains), *L. bozemanii* and *L. dumoffii* (2 strains each), *L. gormanii* and *L. micdadei* (1 strain each), and 4 strains of *Legionella* spp. that were not further identified. These strains were isolated from clinical specimens at Massachusetts General Hospital or referred to our laboratories from other hospitals in the United States.

Standard antibiotic susceptibility powders were gifts from companies as follows: piperacillin and tazobactam from Lederle Laboratories, Pearl River, N.Y.; sulbactam from

Pfizer Inc., Groton, Conn.; lithium clavulanate from Smith-Kline Beecham Pharmaceuticals, Philadelphia, Pa.; cefuroxime from Glaxo Pharmaceuticals, Research Triangle Park, N.C.; cefotaxime from Hoechst-Roussel Pharmaceuticals, Somerville, N.J.; erythromycin from Eli Lilly & Co., Indianapolis, Ind.; clarithromycin from Abbott Laboratories, North Chicago, Ill.; and ciprofloxacin from Miles Pharmaceuticals, West Haven, Conn. Ampicillin and rifampin were purchased from Sigma Chemical Co, St. Louis, Mo.

Antibiotic susceptibility studies were performed by agar dilution in the buffered starch-yeast extract medium described by Saito et al. (12). The *Legionella* spp. strains were thawed from storage at  $-70^\circ\text{C}$  and passed once or twice on buffered charcoal-yeast extract agar (Oxoid USA Inc., Columbia, Md.). From growth after 48 h of incubation, several colonies were taken to prepare bacterial suspensions of the desired cell density in sterile water. Initial suspensions were matched to a 0.5 McFarland standard (expected to be approximately  $10^8$  CFU/ml [1]) and subsequently diluted 1:10 prior to being dispensed into the wells of a multiprong inoculating device. For testing at a higher inoculum, the initial suspension was used undiluted. Actual colony counts were performed for two strain suspensions prepared in this manner and were  $5.2 \times 10^7$  and  $3.6 \times 10^8$  CFU/ml. Final inocula delivered to plates were thus approximately  $1 \times 10^4$  to  $5 \times 10^4$  and  $1 \times 10^5$  to  $5 \times 10^5$  CFU per spot, respectively. *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as controls. Plates were incubated at  $35^\circ\text{C}$  in ambient air and read at 48 and 96 h.

Results of the readings taken at 48 h did not differ from those at 96 h and are shown in Table 1. At the 48-h time point, MICs for the control strains fell within acceptable ranges by National Committee for Clinical Laboratory Standards standards where published (7) or otherwise were comparable to reported MICs of clavulanate alone (10) or MICs of sulbactam alone previously obtained in our own laboratory. At the lower inoculum, piperacillin, tazobactam, and their combination showed equivalent activities against the *Legionella* spp. However, when the inoculum was increased approximately 10-fold, a substantial reduction in the activity of piperacillin alone was noted, while the activ-

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TABLE 1. Comparative in vitro activities of piperacillin-tazobactam and other agents against clinical isolates of *Legionella* species

Antimicrobial agent	MIC ( $\mu\text{g/ml}$ ) for:					
	Lower inoculum <sup>a</sup>			Higher inoculum <sup>b</sup>		
	Range	50%	90%	Range	50%	90%
Piperacillin-tazobactam (8:1) <sup>c</sup>	0.03–0.5	0.125	0.5	0.03–2	0.5	1
Piperacillin	0.03–1	0.125	0.25	0.25–32	4	16
Tazobactam	0.06–2	0.125	0.5	0.125–2	0.5	1
Sulbactam	ND	ND	ND	0.125–8	0.5	2
Clavulanate	ND	ND	ND	0.25–1	0.25	1
Ampicillin	$\leq 0.015$ –4	0.25	2	0.03–16	1	2
Cefuroxime	0.125–4	1	2	$\leq 0.015$ –8	2	4
Cefotaxime	$\leq 0.015$ –1	0.25	0.5	$\leq 0.015$ –2	0.25	0.5
Erythromycin	$\leq 0.015$ –0.25	0.125	0.25	0.03–0.25	0.125	0.25
Clarithromycin	$\leq 0.015$	$\leq 0.015$	$\leq 0.015$	$\leq 0.015$ –0.06	0.03	0.03
Ciprofloxacin	$\leq 0.015$ –0.06	0.03	0.03	$\leq 0.015$ –0.03	0.03	0.03
Rifampin	$\leq 0.015$	$\leq 0.015$	$\leq 0.015$	$\leq 0.015$	$\leq 0.015$	$\leq 0.015$

<sup>a</sup> Approximately  $10^4$  CFU per spot; 28 strains evaluable. 50% and 90%, MICs for 50 and 90% of strains tested; ND, not done.

<sup>b</sup> Approximately  $10^5$  CFU per spot; 29 strains evaluable for tazobactam, piperacillin, and the combination; 25 strains evaluable for other agents.

<sup>c</sup> Expressed as MIC of piperacillin.

ities of tazobactam alone and of the combination were minimally affected. Inhibitory activities of the non- $\beta$ -lactam antimicrobial agents and of cefotaxime, cefuroxime, and ampicillin were virtually identical at the two inocula. MICs of these compounds were comparable to those in previously published studies (4, 8, 9). Ruchdeschel et al. (11) noted significant detrimental effects of increasing the inoculum size 100-fold for most of the  $\beta$ -lactam antibiotics they examined against *Legionella* spp. Specifically, they found geometric mean MICs of ampicillin and piperacillin at the higher inoculum ( $10^6$  CFU) to be increased 8- to 10-fold, while smaller (1.3- and 2.5-fold) but statistically significant increases were noted for cefotaxime and cefuroxime MICs, respectively.

At either inoculum, rifampin, ciprofloxacin, and clarithromycin were each at least eightfold more active than erythromycin on the basis of MICs for 90% of strains tested. MICs of these drugs were within ranges reported previously (1, 2, 4–6). The MIC range and MIC of sulbactam for 50% of strains tested were two- to fourfold lower in our study than previously reported for this drug when tested against *Legionella* spp. in buffered charcoal-yeast agar medium (5).

In the present study, the in vitro activity of the  $\beta$ -lactamase inhibitor tazobactam was equivalent to that of clavulanic acid against the *Legionella* spp., and results with the latter compound were consistent with previous reports (9, 15). Both in tissue culture (16) and in a weanling rat model of infection (13, 14), clavulanic acid has proven effective in reducing numbers of intracellular *L. pneumophila* organisms. In these studies, neither amoxicillin nor ticarcillin alone showed activity against cell-associated organisms. Furthermore, in an animal model, the combination of clavulanate with either amoxicillin or ticarcillin proved comparable to erythromycin in eradication of organisms within pulmonary alveolar macrophages (13, 14). A small but significant advantage was noted for the combination of ticarcillin with clavulanic acid compared with clavulanate alone (13).

Both as a single agent and in combination with piperacillin, tazobactam demonstrated in vitro activity against *Legionella* spp. comparable to that of clavulanic acid. In view of the fact that clavulanic acid, unlike many other  $\beta$ -lactams examined, has shown activity against intracellular *L. pneu-*

*mophila* both in vitro and in vivo, it would be of great interest to examine tazobactam alone and in combination with piperacillin in these systems. If it could be demonstrated that  $\beta$ -lactam antimicrobial agents, such as piperacillin-tazobactam or ticarcillin-clavulanate, which have broad activity against both community-acquired and nosocomial pathogens also effectively inhibit *Legionella* spp. in vivo and are clinically effective, selection of initial therapy for seriously ill patients with pneumonia might be simplified considerably.

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