

## In Vitro Activity of Tigecycline against *Burkholderia pseudomallei* and *Burkholderia thailandensis*

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**Investigation of the in vitro activity of tigecycline against *Burkholderia pseudomallei* and *Burkholderia thailandensis* revealed that the inhibition zone diameters of tigecycline against all isolates were  $\geq 20$  mm and that the MIC<sub>50</sub> values were 0.5 and 1  $\mu\text{g/ml}$  and the MIC<sub>90</sub> values were 2 and 1.5  $\mu\text{g/ml}$  for *B. pseudomallei* and *B. thailandensis*, respectively.**

*Burkholderia pseudomallei*, a gram-negative bacterium, causes in humans and animals a disease called melioidosis (22). The bacterium is a soil organism found mainly in Southeast Asia and northern Australia. Recently, two distinct biotypes of *B. pseudomallei* strains have been defined based on their ability to assimilate L-arabinose and their difference in pathogenicity (2, 7, 17, 23). Both biotypes have been found in soil of areas where melioidosis is endemic in Thailand (20, 21). The Ara<sup>+</sup> *B. pseudomallei* is much less virulent than Ara<sup>−</sup> *B. pseudomallei* (2, 17). However, Ara<sup>+</sup> *B. pseudomallei* has been reported to cause disease in humans (11). Subsequently, a distinct new species, *Burkholderia thailandensis*, was proposed for the Ara<sup>+</sup> *B. pseudomallei* strain (3). *B. pseudomallei* is usually resistant to many antibiotics. Antibiotics currently recommended for therapy of melioidosis are ceftazidime, imipenem, meropenem, amoxicillin/clavulanate, cefoperazone/sulbactam, trimethoprim-sulfamethoxazole, doxycycline, and chloramphenicol (22). The development of resistance of *B. pseudomallei* to these antibiotics was recognized (6, 8, 19, 24), and hence a search for new agents effective against *B. pseudomallei* is needed.

Tigecycline is a glycylcycline antibiotic that shows promising activity against a wide range of organisms (25). Tigecycline is active against gram-positive cocci, including methicillin-resistant staphylococci, penicillin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant enterococci. Tigecycline is also active against many gram-negative bacilli, including those resistant to multiple antibiotics as well as anaerobes. However, the activity of tigecycline against *B. pseudomallei* has not been reported. The present study was undertaken to explore the activity of tigecycline against *B. pseudomallei* and *B. thailandensis*.

One hundred twenty-six strains of *B. pseudomallei* and *B. thailandensis* were selected from our collection. One hundred two strains of *B. pseudomallei* were isolated from different infected patients, and 24 strains of *B. thailandensis* were isolated from 23 soil samples collected from different sites and from one infected patient. All *Burkholderia* species were identified with the API 20NE (bioMérieux, France). *B. pseudomallei* and *B. thailandensis* were differentiated by the arabinose

assimilation test (20). In vitro susceptibilities were determined by Kirby-Bauer disk diffusion, Etest, and MicroScan. Paper disks containing tigecycline at 15  $\mu\text{g}$  per disk (Becton Dickinson), Etest strips (AB Biodisk), and gram-negative MicroScan MIC panels (Dade Behring Inc.) were provided by Wyeth Research. Susceptibility testing was done by direct colony suspension according to guidelines suggested by CLSI (4). Quality control was performed by testing the susceptibility of *Escherichia coli* ATCC 25922 as recommended by Wyeth Research.

The distribution of inhibition zone diameters of tigecycline against *B. pseudomallei* and *B. thailandensis* is shown in Table 1. All strains had an inhibition zone diameter of  $\geq 20$  mm. The MIC<sub>50</sub> and MIC<sub>90</sub> values of tigecycline as determined by Etest are shown in Table 2. The MIC<sub>50</sub> values were 0.5 and 1  $\mu\text{g/ml}$  for *B. pseudomallei* and *B. thailandensis*, respectively. The MIC<sub>90</sub> values were 2 and 1.5  $\mu\text{g/ml}$  for *B. pseudomallei* and *B. thailandensis*, respectively. There was a significant correlation between inhibition zone diameters and MICs from Etest ( $P < 0.001$ ;  $r = -0.68$ ). The mean inhibition zone diameters of the strains with MIC of 3, 2, 1.5, 1, 0.75, and 0.5  $\mu\text{g/ml}$  were 20, 21.8, 22.6, 23.5, 24.4, and 26.7 mm, respectively. The correlation of MICs determined by Etest and MicroScan was satisfactory, as shown in Table 3. Thirty-two strains (50%) had identical MICs determined by Etest and MicroScan, whereas another 50% had a difference in MICs of 0.25 to 0.5  $\mu\text{g/ml}$ .

The MICs of tigecycline for *B. pseudomallei* and *B. thailandensis* observed in our study were higher than those for *S. pneumoniae*, *Staphylococcus aureus*, *Enterococcus* spp., and non-ESBL-producing *Enterobacteriaceae* (1). However, they were comparable to the MICs of tigecycline for *Acinetobacter* spp., *Enterobacter aerogenes*, and ESBL-producing *Klebsiella pneumoniae* (1). The breakpoints for inhibition zone diameter and MIC of tigecycline against *B. pseudomallei* and *B. thailandensis* are not available. The breakpoint of doxycycline against *B. pseudomallei*, adapted from data compiled by the National Committee for Clinical Laboratory Standards for similar organisms to be used for susceptibility testing, was 4  $\mu\text{g/ml}$  (15). The U.S. FDA-approved breakpoints of tigecycline against *Enterobacteriaceae* to be used by local laboratories were an inhibition zone diameter of  $\geq 19$  mm and a MIC of  $\leq 2$   $\mu\text{g/ml}$  (4). With the aforementioned breakpoints used to determine susceptibility of *Burkholderia* spp. to tigecycline, all isolates of *B. pseudomallei* and *B. thailandensis* were susceptible to tige-

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TABLE 1. Distribution of tigecycline susceptibility as determined by disk diffusion for *B. pseudomallei* and *B. thailandensis*

Organism (no. of isolates)	No. (%) of isolates for which the inhibition zone diameter (mm) was:									
	20	21	22	23	24	25	26	27	28	30
<i>B. pseudomallei</i> (102)	12 (11.8)	16 (15.7)	26 (25.5)	21 (20.6)	2 (2)	24 (23.5)				1 (1)
<i>B. thailandensis</i> (24)		1 (4.2)	3 (12.5)	5 (20.8)		13 (54.2)	1 (4.2)			1 (4.2)

cycline according to the inhibition zone diameter criteria and 98% of *B. pseudomallei* isolates and all *B. thailandensis* isolates were susceptible to tigecycline according to the MIC criteria. The MICs determined by Etest were significantly correlated with the inhibition zone diameters and the MICs determined by MicroScan. Therefore, disk diffusion and Etest methods are reliable for determination of susceptibility of tigecycline against *B. pseudomallei* and *B. thailandensis*.

Pharmacokinetic and pharmacodynamic studies of tigecycline in healthy subjects after a 100-mg loading dose given intravenously followed by 50 mg every 12 h have been reported (5, 12–14, 18). The mean maximum concentration ( $C_{\max}$ ), the mean time to maximum concentration, the mean minimum concentration ( $C_{\min}$ ), the mean area under the curve, and the mean half-life of tigecycline in serum were 0.72  $\mu\text{g/ml}$ , 0.52 h, 0.1  $\mu\text{g/ml}$ , 1.73  $\mu\text{g} \cdot \text{h/ml}$ , and 15 h, respectively. These profiles were favorable for many organisms, such as *S. pneumoniae*, *Chlamydia pneumoniae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, and *Haemophilus influenzae*, since the MIC<sub>90</sub> values of tigecycline for such organisms were very low. The mean  $C_{\max}$  of tigecycline in serum (0.72  $\mu\text{g/ml}$ ) after the conventional dose of tigecycline (100-mg loading dose followed by 50 mg every 12 h) was above the MICs of tigecycline for only 6% of *B. pseudomallei* and *B. thailandensis* isolates. However, it was found that tigecycline had a large volume of distribution (7 to 10 liters/kg), indicating extensive distribution into the tissues (13). In addition, the mean  $C_{\max}$ , the mean time to maximum concentration, the mean  $C_{\min}$ , the mean area under the curve, and the mean half-life of tigecycline in the alveolar cells were 15.2  $\mu\text{g/ml}$ , 2 h, 6.4  $\mu\text{g/ml}$ , 134  $\mu\text{g} \cdot \text{h/ml}$ ,

and 23.7 h, respectively (5). These observations imply that tigecycline accumulates in the cells and could be effective for infections caused by intracellular organisms. The mean  $C_{\max}$  and the mean  $C_{\min}$  of tigecycline in the alveolar cells were much higher than the MICs for all isolates of *B. pseudomallei* and *B. thailandensis*. Therefore, tigecycline should be a suitable antibiotic for therapy of melioidosis, since *B. pseudomallei* is an intracellular bacterium (9, 10, 16). This hypothesis needs further investigation by conducting clinical trials on therapy of melioidosis with tigecycline.

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TABLE 2. MICs of tigecycline for *B. pseudomallei* and *B. thailandensis* as determined by Etest

Organism (no. of isolates)	MIC ( $\mu\text{g/ml}$ )		
	Range	For 50% of isolates	For 90% of isolates
<i>B. pseudomallei</i> (102)	0.5–3	0.5	2
<i>B. thailandensis</i> (24)	0.5–1.5	1	1.5

TABLE 3. Correlation between MICs determined by Etest and MicroScan

MIC ( $\mu\text{g/ml}$ ) determined by MicroScan	No. of strains with the following MIC ( $\mu\text{g/ml}$ ) determined by Etest:				
	0.50	0.75	1.00	1.50	2.00
0.50	1	1	1		
0.75					
1.00	1	2	22	24	
1.50					
2.00				3	9

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