In Vitro Activity of Doripenem against *Burkholderia pseudomallei*

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The MIC$_{50}$ and MIC$_{90}$ values of doripenem, determined by Etest, for 110 isolates of *Burkholderia pseudomallei* were 0.5 and 0.75 μg/ml, respectively. There were significant correlations between MICs determined by Etest and MICs determined by agar dilution, MICs determined by Etest and inhibition zone size, and MICs determined by agar dilution and inhibition zone size.

*Burkholderia pseudomallei*, a gram-negative bacterium, causes a disease called melioidosis in humans and animals (13). *B. pseudomallei* is usually resistant to many antibiotics. Antibiotics currently recommended as therapy for melioidosis are ceftazidime, imipenem, meropenem, amoxicillin-clavulanate, cefoperazone-sulbactam, trimethoprim-sulfamethoxazole, doxycycline, and chloramphenicol (13). The development of resistance by *B. pseudomallei* to the aforementioned antibiotics was recognized (3, 4, 10, 11, 14); hence, a search for new agents effective against *B. pseudomallei* is needed.

Doripenem is a new parental 1-β-methyl carbapenem. Doripenem has demonstrated activity against a wide range of gram-positive and gram-negative bacteria, including *Pseudomonas aeruginosa* (7, 8). Against *P. aeruginosa*, doripenem exhibits rapid bactericidal activity, with two- to fourfold-lower MICs than those of meropenem. However, the activity of doripenem against *B. pseudomallei* has not been reported. The present study was undertaken to explore the activity of doripenem against *B. pseudomallei*.

One hundred ten clinical isolates of *B. pseudomallei* from different patients were selected from our collection. All isolates of *B. pseudomallei* were identified by API 20NE (bioMerieux, France). These isolates are susceptible to imipenem and meropenem, according to the inhibition zone diameter criteria for *P. aeruginosa* (≥16 mm). In vitro susceptibilities of all isolates were determined by Etest. Thirty isolates were randomly selected for determination of the MICs for doripenem by using the agar dilution and Kirby-Bauer disk diffusion methods. Paper disks containing doripenem (10 μg per disk; Becton Dickinson), Etest strips (AB Biodisk, Sweden), and doripenem powder (Johnson & Johnson Pharmaceutical Research & Development) were used. The methodology used for susceptibility testing was direct colony suspension, according to guidelines suggested by the CLSI (2). Quality control was performed by testing the susceptibility of *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

The MICs of doripenem for the quality control organisms, namely *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922, were 0.125 and 0.012 μg/ml, respectively. Both values were within the reference limits. A distribution of the MICs for doripenem determined by Etest for 110 strains of *B. pseudomallei* is shown in Table 1. Etest results showed doripenem MICs ranging from 0.19 to 2 μg/ml, and the MIC$_{50}$ and the MIC$_{90}$ values were 0.5 and 0.75 μg/ml, respectively. The MIC$_{50}$ and MIC$_{90}$ values of doripenem, determined by agar dilution for 30 strains of *B. pseudomallei*, were 1 and 1.5 μg/ml, respectively. The inhibition zone diameters of doripenem determined for 30 strains of *B. pseudomallei* ranged from 24 to 36 mm. There was a significant correlation between the MICs determined by Etest and the MICs determined by agar dilution (r of 0.9; P of <0.001), the MICs determined by Etest and the inhibition zone diameters (r of −0.7; P of <0.001), and the MICs determined by agar dilution and the inhibition zone diameters (r of −0.7; P of <0.001), as shown in Fig. 1.

The MIC breakpoint recommended by the CLSI for imipenem-susceptible *B. pseudomallei*, imipenem-resistant *P. aeruginosa*, and meropenem-susceptible *P. aeruginosa* is ≤4 μg/ml. The MIC breakpoint recommended by the FDA for imipenem-susceptible *P. aeruginosa* and meropenem-susceptible *P. aeruginosa* is also ≤4 μg/ml. The MIC breakpoint recommended by the CLSI for meropenem-susceptible *Burkholderia cepacia* is ≤4 μg/ml. However, the breakpoints for the MIC and inhibition zone diameter of doripenem for *B. pseudomallei* are not available. The U.S. FDA-approved breakpoints of doripenem for *P. aeruginosa* were a MIC of ≤2 μg/ml and an inhibition zone diameter of ≥24 mm. If the aforementioned breakpoints were used to determine susceptibility of *B. pseudomallei* to doripenem, all isolates of *B. pseudomallei* are considered susceptible to doripenem, according to inhibition zone diameter and MICs determined by Etest; more than 90% of *B. pseudomallei* isolates are considered susceptible to doripenem, according to MICs determined by agar dilution. The susceptibility profiles of *B. pseudomallei* to imipenem and

<p>| Table 1. Distribution of the MICs for doripenem determined by Etest for 110 isolates of <em>B. pseudomallei</em> |</p>
<table>
<thead>
<tr>
<th>No. of isolates (%)</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (1.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>2 (1.8)</td>
<td>0.25</td>
</tr>
<tr>
<td>32 (29.1)</td>
<td>0.38</td>
</tr>
<tr>
<td>60 (54.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>13 (11.8)</td>
<td>0.75</td>
</tr>
<tr>
<td>1 (0.9)</td>
<td>2</td>
</tr>
</tbody>
</table>

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meropenem were reported (5, 6, 10, 11). The MIC$_{50}$ and MIC$_{90}$ values for imipenem against *B. pseudomallei* were 1 and 1 µg/ml by agar dilution, respectively, and 0.5 and 1 µg/ml by Etest, respectively. The MIC$_{50}$ and MIC$_{90}$ values for meropenem against *B. pseudomallei* were 3 and 4 µg/ml by broth microdilution, respectively, and 1 and 2 µg/ml by Etest, respectively. Therefore, the susceptibility profiles of *B. pseudomallei* to doripenem are comparable to those of *B. pseudomallei* to imipenem and meropenem.

The inhibition zone diameters of doripenem for *B. pseudomallei* were significantly correlated with the MICs determined by Etest or agar dilution. Therefore, Kirby-Bauer disk diffusion could be used to determine the in vitro activity of doripenem against *B. pseudomallei*. However, the MICs of doripenem determined by agar dilution for *B. pseudomallei* observed in our study were twofold higher than those determined by Etest. Nevertheless, the MIC$_{90}$ of doripenem determined by agar dilution was $\leq$2 µg/ml.

Ceftazidime is the conventional agent used as therapy for acute severe melioidosis (12). Imipenem and meropenem were also found to be effective and safe when used as therapy for acute severe melioidosis (1, 9), and they are considered to be alternative therapeutic agents (15). Since the in vitro activity of doripenem against *B. pseudomallei* is comparable to the activity of imipenem and meropenem against *B. pseudomallei*, doripenem should be an effective therapy for melioidosis. However, a clinical trial is required to fully establish the efficacy and safety of doripenem used as therapy for melioidosis.

Johnson & Johnson Pharmaceutical Research & Development provided the doripenem susceptibility disks, Etest strips, and powder for this study.

REFERENCES

![Graph A](image1.png)

FIG. 1. Correlations between the MICs determined by Etest and agar dilution and the inhibition zone diameters. (A) Correlation between the MICs determined by Etest and the MICs determined by agar dilution. (B) Correlation between the MICs determined by Etest and the inhibition zone diameters. (C) Correlation between the MICs determined by agar dilution and the inhibition zone diameters.


