Bacteriostatic and Bactericidal Activities of Tigecycline against *Coxiella burnetii* and Comparison with Those of Six Other Antibiotics

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The present article is a study of the in vitro susceptibility of eight Greek *Coxiella burnetii* isolates, derived from patients with acute Q fever, and two reference strains of *Coxiella burnetii* to tigecycline. The bacteriostatic activity of tigecycline was compared with those of six other antibiotics using a shell vial assay. The MICs of the examined antibiotics were as follows: tigecycline ranged from 0.25 to 0.5 μg/ml; doxycycline, trovafloxacin, and ofloxacin ranged from 1 to 2 μg/ml; linezolid and clarithromycin ranged from 2 to 4 μg/ml; and ciprofloxacin ranged from 4 to 8 μg/ml. Tigecycline was effective in inhibiting the infection of Vero cells by *C. burnetii*. No bactericidal activity was observed against *C. burnetii* at 4 μg/ml.

*Coxiella burnetii*, a strict intracellular bacterium, is the etiologic agent of Q fever. Two major forms of the disease are known. The acute form is usually a self-limiting, febrile illness, during which pneumonia or hepatitis may occur. The chronic form is a severe disease in which endocarditis predominates (15). Although acute *C. burnetii* infections respond relatively well to antibiotic therapy, chronic infections constitute a therapeutic problem (7, 14, 20). Hence, in the case of chronic Q fever, bactericidal activity is required to eradicate *C. burnetii* (10, 14). Tetracyclines remain the first-line antibiotics to treat Q fever. However, the isolation of viable *C. burnetii* bacteria from valve tissue after 4 years of therapy with doxycycline has been reported (14). Tigecycline, a tetracycline derivative, belongs to a novel class of antibiotics known as the glycy cyclines. In vitro and in vivo studies have shown that tigecycline possesses broad-spectrum antibacterial activity against extracellular gram-negative, gram-positive, and anaerobic bacteria (11). In addition, the intracellular penetration of tigecycline may play a critical role in inhibiting the intracellular multiplication of bacteria, thus treating such infections (3). An abundance of data has been accumulated on the antimicrobial activity of tigecycline and on the mechanisms responsible for increased susceptibility to tigecycline (11). The efficacy of tigecycline against the acute or chronic form of Q fever infection has yet to be established.

In this paper, we report the activity of tigecycline against the Nine-Mile and Q212 reference strains of *C. burnetii*, as well as eight Greek isolates derived from patients with acute Q fever (19). In addition, we compared the bacteriostatic effect of tigecycline with that of six other antibiotic compounds on the same strains.

All strains were cultured in Vero cells as previously described (4, 5, 13, 19). A stock solution of clarithromycin (6 mg/ml; Abbott Laboratories, Chicago, IL) was prepared using methanol. Linezolid (200 mg/100 ml; Pharmacia and Upjohn, Inc., NJ), tigecycline (50 mg; Wyeth, NJ), ciprofloxacin (100 mg/50 ml; Bayer AG, Leverkusen, Germany), and ofloxacin (220 mg/100 ml; Hoechst AG, Frankfurt am Main, Germany) for injection were also used in this study. For trovafloxacin (20 mg/ml; Pfizer, Inc., NY) and doxycycline (10 mg/ml; Pfizer, Inc., NY), the stock solution was prepared with sterile distilled water. All antibiotics were divided into aliquots and frozen at −20°C. The aliquots were quickly thawed, and the drug solution was incorporated into minimal essential medium for the antibiotic challenges. At the concentrations used (for tigecycline, 0.03 μg/ml to 8 μg/ml, and for the rest of the antibiotics, 0.25 μg/ml to 16 μg/ml), the solvents were nontoxic to normal or infected Vero cells.

The bacteriostatic and bactericidal effects of antibiotics against *C. burnetii* were tested by a shell vial assay (1, 4, 5, 6, 9, 13, 16, 21). For this purpose, Vero cell monolayers cultured in shell vials were infected with 10-fold serial dilutions of a *C. burnetii* inoculum to determine the inoculum dilution that resulted in 50% infected Vero cells after 6 days of incubation. For each isolate, the effect of an antibiotic compound was assessed by comparing the shell vials that were treated with those not treated with antibiotic (positive control). Bacterial growth was evaluated after 6 days of incubation by indirect immunofluorescence, and the results were scored as follows: R for resistance or infection under antibiotic treatment comparable to that of the positive nondrug control (normal growth or 50% infected cells); I for intermediate susceptibility (decreased growth or less than 10% infected cells); and S for susceptibility, the absence of infected cells, or the presence of isolated bacteria (no growth) (13). Briefly, to determine the bacteriostatic effect, the activities of the antibiotics were evaluated by their capacity to inhibit *C. burnetii* growth in shell vials compared to that of a drug-free control leading to 50% of the infected cell monolayers after 6 days of incubation. The bactericidal activity of tigecycline was assessed by the quantitative...
strains for 11 months. In the method described by Maurin and Raoult (8, 9). Vero cells were persistently infected with the Nine-Mile and Q212 \textit{C. burnetii} strains for 11 months. In the \textit{C. burnetii} cultures described above, tigecycline was added to the culture medium in different concentrations ranging between 0.5 and 4 \( \mu g/mL \). Flasks without antibiotic, containing Vero cells persistently infected with the reference strains, served as negative controls. All flasks were then incubated for 24 h at 37°C in a 5% \( CO_2 \) atmosphere. Bactericidal activity corresponded to a significant reduction in bacterial titer (at least 2 to 3 dilutions) after antibiotic exposure, compared with that in the primary inoculum dose (4, 5, 8). All experiments were performed in duplicate and repeated to confirm results.

Table 1 summarizes the susceptibilities of all the \textit{C. burnetii} strains to the antibiotics tested. Tigecycline showed no evidence of toxicity with the Vero cells, for concentrations up to 4 \( \mu g/mL \) in the drug-only control flasks. The results for the MICs in the two independent experiments were identical. Tigecycline showed bacteriostatic effects against the \textit{C. burnetii} species and \textit{Rickettsia} strains were used (11). The order of the efficacy of the antibiotics used in this study against \textit{C. burnetii} Greek isolates and \textit{C. burnetii} reference strains was tigecycline > doxycycline, ofloxacin, trovafloxacin > clarithromycin, linezolid > ciprofloxacin.

An evaluation of the antibiotic susceptibility of \textit{C. burnetii} isolates is hazardous and time consuming to be performed in routine laboratories. However, in cases of chronic Q fever, it will be important to determine the antibiotic susceptibility of the agent, since chemotherapy is long lasting and relapses may occur (15).

In antibiotics exhibiting concentration-dependent bactericidal effects, such as fluoroquinolones, the ratio of concentration to MIC is a better predictor of bacterial growth inhibition in the case of extracellular microorganisms and subsequently of clinical efficacy. Furthermore, in the case of time-dependent antibiotics such as penicillins, macrolides, and clindamycin, the ratio of time to MIC (T/MIC) predicts the efficacy of the antibiotic against extracellular microorganisms (2). Conversely, only MICs are used to compare the efficacy of antibiotics in cases of intracellular organisms. In conclusion, our results indicate that tigecycline possesses enhanced in vitro activity against \textit{C. burnetii} and may be a potential alternative to doxycycline in cases of acute Q fever. Additionally, the absence of the bactericidal activity of this compound against \textit{C. burnetii} does not substantiate a therapeutic benefit in using this drug to treat patients with chronic Q fever. Clinical trials are needed for the evaluation of the clinical usefulness of tigecycline.

\textbf{TABLE 1. Susceptibilities of \textit{C. burnetii} isolates to seven antibiotics}\n
<table>
<thead>
<tr>
<th>Isolate</th>
<th>Doxycycline</th>
<th>Linezolid</th>
<th>Trovafloxacin</th>
<th>Ofloxacin</th>
<th>Clarithromycin</th>
<th>Ciprofloxacin</th>
<th>Tigecycline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>R</td>
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<tr>
<td>Nine-Mile</td>
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<td>1</td>
<td>2</td>
<td>4</td>
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</tr>
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<td>CP1</td>
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<td>1</td>
<td>2</td>
<td>4</td>
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</tr>
<tr>
<td>CP2</td>
<td>0.25</td>
<td>0.5</td>
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<td>0.5</td>
<td>1</td>
<td>2</td>
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<tr>
<td>CP3</td>
<td>0.25</td>
<td>0.5</td>
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<td>0.5</td>
<td>1</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
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<td>2</td>
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<td>1</td>
<td>2</td>
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<tr>
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<td>0.5</td>
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<td>1</td>
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<tr>
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<tr>
<td>CP8</td>
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<td>0.5</td>
<td>1</td>
<td>2</td>
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</tr>
</tbody>
</table>

* R, resistant; I, intermediate; S, susceptible.
REFERENCES