Mechanisms That May Account for Differential Antibiotic Susceptibilities among Coxiella burnetii Isolates

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The Nine Mile, S Q217, and Priscilla isolates, representative of the three major genetic groups of Coxiella burnetii, are known to differ in their susceptibilities to antibiotics. Mechanisms potentially responsible for these differences were investigated. Accumulation of antibiotics by infected L929 cells and purified isolates was measured. In addition, C. burnetii plasmid-transformed Escherichia coli HB101 cells were used to study the possibility that different C. burnetii plasmids are responsible for disparate antibiotic susceptibilities of the isolates. L929 cells recently or persistently infected with the Priscilla isolate exhibited a significantly reduced accumulation of [³H]tetracycline as compared with that in L929 cells infected with either the Nine Mile or S Q217 isolates; accumulation of this drug was greater in cells recently infected with each isolate. In contrast, L929 cells recently or persistently infected with the different isolates accumulated [³H]norfloroxacin to an equivalent extent. [³H]Tetracycline accumulation was approximately the same among the purified isolates. However, as measured by both scintillation and spectrofluorometry, norfloroxacin accumulation was significantly diminished in the purified Priscilla isolate. pH had no apparent effect upon isolate permeabilities. The presence of C. burnetii QpH1 or QpRS plasmids did not alter the antibiotic susceptibility of E. coli. Collectively, these results indicate that differential susceptibilities to tetracyclines or fluoroquinolones in C. burnetii isolates may be the result of distinct mechanisms involving altered host-cell (tetracyclines) or isolate-specific (fluoroquinolones) permeabilities.

Recent reports indicate that Coxiella burnetii isolates are genetically distinct and that different isolates may be associated with distinct clinical forms of Q fever—acute versus chronic (13, 23). Furthermore, the Nine Mile (NM; RSA 493) and Priscilla (PRS; Q177) isolates, implicated in acute and chronic disease syndromes, respectively, exhibit dramatically different susceptibilities to a variety of antibiotics, including doxycycline, rifampin, and several fluoroquinolones (31, 32). These observations correlate with clinical success in treating acute Q fever and the poor antibiotic manageability of chronically diseased patients (29). Increased optimism for a more effective treatment of the chronic disease syndrome has been generated by the recent report (34) that ciprofloxacin, a fluoroquinolone antibiotic, has been effective in controlling human chronic Q-fever endocarditis. Other workers have recently reported the failure of this drug to sterilize heart valves (20). Previously, other investigators (26) have demonstrated tetracycline resistance in one C. burnetii isolate (Cyprus). Obligate intracellular pathogens like C. burnetii present special problems in antibiotic resistance: unless drugs can penetrate into phagocytic cells and accumulate in active forms within phagolysosomes, they will be ineffective against such organisms.

The antibiotic susceptibility of C. burnetii isolates, however, is probably not the only factor determining success in patient treatment. In contrast to clinical results, the S Q217 isolate exhibits unexpectedly high antibiotic susceptibilities in vitro (31). Such results suggest that a patient predisposition factor is partly involved in the development of the chronic disease syndrome.

The drugs to which the three C. burnetii isolates studied (NM, S Q217, and PRS) exhibited differential susceptibilities (30-33) have different targets and modes of action (19). It is therefore unlikely that drug-specific mutational alterations account for the differential susceptibilities of the isolates to doxycycline, rifampin, or the fluoroquinolones. Rather, a more broad resistance mechanism, such as decreased permeability or increased efflux of drugs, likely underlies the variability in antibiotic susceptibility observed among the isolates. The possibility that growth rate variation accounts for differences in antibiotic susceptibility has not been ruled out.

In this report, we present results of experiments designed to investigate mechanisms that may account for differential antibiotic susceptibilities observed among C. burnetii isolates. In addition, experiments were performed to examine the possibility that C. burnetii plasmids mediate differential antibiotic susceptibilities. Evidence is presented that indicates that permeability differences among infected host cells and/or the isolates themselves underlie these differences. Furthermore, C. burnetii plasmids do not appear to be directly involved in determining antibiotic susceptibility.

MATERIALS AND METHODS

Source and in vitro propagation of C. burnetii isolates. The phase I C. burnetii NM isolate was obtained from M. Peacock of the Rocky Mountain Laboratory, Hamilton, Mont. The S Q217 and PRS isolates were provided by L. Mallaviala, Washington State University, Pullman. All organisms were obtained in yolk sac homogenates and propagated within L929 mouse fibroblast cell cultures (21). Recent infections were initiated and maintained as described previously (32) and were used in experiments at ≤30 days

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postinfection; L929 cells infected for ≥100 days were designated as persistently infected.

C. burnetii-plasmid Escherichia coli HB101 transductants. E. coli HB101 organisms transduced with the NM or PRS C. burnetii isolate plasmid (QpH1 or QpRS, respectively) were obtained from L. Mallavia. The plasmids were used as vectors within the pHK17 cosmid. The three E. coli HB101 transductants used were designated pHK17 (control, lacking C. burnetii sequences), pHK17::QpH1 (NM), and pHK17::QpRS (PRS). The pHK17 vector contained a kanamycin resistance gene; all E. coli transductants were cultured in Luria-Bertani (LB) medium (pH 7.5; 15 containing 50 μg of kanamycin sulfate (Sigma Chemical Co., St. Louis, Mo.) per ml. Broth cultures were incubated at 37°C for 18 to 24 h with vigorous agitation to ensure aeration.

Antibiotic preparation and use. Ciprofloxacin was provided by B. Painter, Miles Pharmaceuticals, West Haven, Conn., and prepared as previously described (31). [3H] norfloxacin (specific activity, 1,540 mCi/mmol) and [3H]tetraacycline (specific activity, 500 mCi/mmol) were generously provided by L. Shen (Abbott Laboratories, North Chicago, Ill.) and W. C. Buss (Department of Pharmacology, University of New Mexico School of Medicine, Albuquerque), respectively. Both radioactive antibiotics were obtained in solution previously determined to be 99% pure. Cultures were then pelleted, and the fluorescence of the supernatant fluid was determined at 525 nm (excitation wavelength, 431 nm).

Antibiotic disk diffusion assays of E. coli transductants. Antibiotic susceptibilities of E. coli HB101 transductants with or without C. burnetii plasmids were determined by using Kirby-Bauer-type disk diffusion assays (3). Ultrapure paper filter disks (12.7 mm; Schleicher & Schuell, Inc., Keene, N.H.) were impregnated with antibiotics and placed on LB-kanamycin agar plates confluently inoculated with E. coli transductants before incubation (24 h, 37°C). Zones of inhibition of the three transductant strains (pHK17 control, pHK17::QpH1, and pHK17::QpRS) were then compared. This method of assay was also used to determine the susceptibilities of E. coli transductants treated with ciprofloxacin to deplete C. burnetii plasmids (see below).

RESULTS

In these studies, we investigated possible mechanisms underlying variable antibiotic susceptibilities among C. burnetii isolates. Previously (30–32), we found that isolates of C. burnetii implicated in distinct Q-fever syndromes exhibited very different susceptibilities to rifampin, tetracyclines, and fluorquinolones. Isolates implicated in chronic disease have consistently been significantly more resistant to a variety of antibiotics than those implicated in acute disease only.

Ciprofloxacin-induced plasmid depletion of E. coli transductants. The methods used in determining the presence of plasmids in E. coli transductants were similar to those used by Weisser and Wiedemann (27) and others (17, 18). E. coli antibiotic susceptibilities were determined before and after C. burnetii plasmid depletion to establish the role of the plasmids in effecting drug susceptibility. E. coli transductants grown in LB-kanamycin broth (37°C for 24 h, 200 rpm) with or without 0.006 μg of ciprofloxacin per ml were serially diluted and plated on LB agar with or without kanamycin (50 μg/ml). Colonies were enumerated after incubation (24 h, 37°C). Organisms depleted of the pHK17 vector were presumably unable to grow on LB agar containing kanamycin. Comparison of growth on LB media with and without kanamycin allowed quantification of plasmids.
or persistently (>100 days) infected with NM, S Q217, or PRS C. burnetii isolates were used to investigate infected host cell permeability to [3H]tetracycline or [3H]norfloxacin. L929 cells were suspended in culture medium (SMEM) during experimentation to maintain typical culture conditions. Uptake of [14C]-amino acids verified L929 cell viability throughout experimentation (Fig. 1 and 2). L929 cells persistently infected with the NM isolate exhibited a significantly higher level of [3H]tetracycline accumulation than did cells infected with either the S Q217 or PRS isolate (Fig. 1). Furthermore, L929 cells recently infected (22 days) with these isolates accumulated more [3H]tetracycline than did their persistently infected (>100 days) counterparts after 60 min of incubation (Fig. 3). In contrast, L929 cells infected with the different C. burnetii isolates exhibited no significant difference in their accumulation of [3H]norfloxacin (Fig. 2). In addition, recent or persistent infection had no detectable effect on L929 cell [3H]norfloxacin accumulation (data not shown).

Antibiotic accumulation studies of purified C. burnetii isolates. NM, S Q217, and PRS isolates of C. burnetii were purified from persistently infected L929 cells and tested for their accumulation of [3H]tetracycline or [3H]norfloxacin at pH 4.5 and 7.5. C. burnetii has been shown to be metabolically active at acidic pH but inactive at neutral pH (11). Moreover, C. burnetii is known to reside and proliferate within acidic phagolysosomes (2). The three isolates accumulated similar levels of radioactive tetracycline at both pH 4.5 and 7.5 (Fig. 4 and 5). However, the PRS isolate accumulated significantly less [3H]norfloxacin than did either the NM or S Q217 isolate (Fig. 6 and 7) at either pH 4.5 or 7.5; spectrofluorometric norfloxacin accumulation assays confirmed these findings (Fig. 8 and 9).

Antibiotic disk diffusion assays of E. coli transductants. Kirby-Bauer-type antibiotic disk diffusion assays (3) were employed to test the possibility that C. burnetii plasmids confer drug resistance. A susceptibility comparison of E. coli HB101 containing no C. burnetii plasmid (pHK17 control) or with NM (pHK17::QpH1) or PRS (pHK17::QpRS) C. burnetii plasmid-transduced organisms was performed.

FIG. 1. Relative accumulation of [3H]tetracycline by L929 fibroblasts persistently infected (over 250 days) with different isolates of C. burnetii: NM (○), S Q217 (□), or PRS (△). Tetracycline accumulation in normal, uninfected L929 cells (●) is shown for comparison. All C. burnetii isolates were phase 1 at time of initial L929 cell infection. Linear regression analyses were performed on data from four independent experiments with similar results; the linear correlation coefficient was >0.5 (two-tailed test; P < 0.03). The dashed line indicates [14C]-amino acid accumulation by L929 cells during the experiment.

FIG. 2. Relative accumulation of [3H]norfloxacin by L929 fibroblasts persistently infected (over 250 days) with different isolates of C. burnetii: NM (○), S Q217 (□), or PRS (△). Norfloxacin accumulation in normal, uninfected L929 cells (●) is shown for comparison. All C. burnetii isolates were phase 1 at time of initial L929 cell infection. Linear regression analyses were performed on data from three independent experiments with similar results; the linear correlation coefficient was >0.5 (two-tailed test; P < 0.05). The dashed line indicates [14C]-amino acid accumulation by L929 cells during experimentation.

FIG. 3. Comparison of [3H]tetracycline accumulation between L929 cells recently (22 days) and persistently (>100 days) infected with the NM isolate of C. burnetii. These data represent results from three independent experiments. These differences were approximately equal to those found between L929 cells recently and persistently infected with the S Q217 and PRS isolates.
with a variety of antibiotics, including several quinolones, tetracyclines, aminoglycosides, rifampin, chloramphenicol, nalidixic acid, and several drug combinations. These drugs have been previously shown to possess differential efficacies against the NM, S Q217, and PRS isolates (30-32). The antibiotic susceptibilities of C. burnetii plasmid-transduced E. coli HB101 isolates were also determined for transductants treated with ciprofloxacin to eliminate plasmids (Table 1). In no case did the presence of either the QpH1 (NM) or

DISCUSSION

Distinct isolates of C. burnetii have been shown to exhibit significant differences in antibiotic susceptibility in vitro (30-32) and may be responsible for the distinct clinical disease syndromes of Q fever observed in vivo (13, 23). The broad in vitro antibiotic resistance characteristic of the PRS isolate, implicated in chronic Q fever, correlates with the poor results in treating chronically infected patients with antibiotics (29). Recent evidence indicates that, as predicted by in vitro studies (29-32), fluoroquinolone antibiotic therapy may be effective in treating chronic Q-fever endocarditis
susceptibilities were greater for fibroblasts than for P-25 buffer measured by spectrofluorometry (excitation wavelength, 317 nm; emission wavelength, 525 nm). Isolates were purified from L929 fibroblasts persistently infected for over 250 days and suspended in modified P-25 buffer (11). The norfloxacin concentration was 5 μg/ml. Data represent mean values of independent, duplicate experiments with very similar results.

Unexpectedly, the S Q217 C. burnetii isolate, recovered from a chronic Q-fever endocarditis patient for whom long-term antibiotic therapy was ineffective, exhibited much greater susceptibilities to antibiotics in vitro than did the PRS isolate (30). The patient from whom the S Q217 isolate was recovered had undergone combination rifampin and doxycycline therapy for many months apparently without cure; relapse occurred after cessation of drug treatment (30). This implies that other factors, including immunological, are involved in the establishment and maintenance of the chronic Q-fever disease syndrome.

The NM and PRS isolates possess distinct plasmids (13, 22, 23); the S Q217 isolate lacks free plasmid DNA (although it contains plasmid DNA integrated into the chromosome

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**TABLE 1. Effect of ciprofloxacin treatment on plasmid stability in E. coli HB101 transductants containing C. burnetii QpH1 (NM) or QpRS (PRS) plasmids**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>CIP− KAN−</th>
<th>CIP+ KAN−</th>
<th>CIP+ KAN+</th>
<th>% Loss&lt;sub&gt;CIP&lt;/sub&gt;</th>
<th>% Loss&lt;sub&gt;α&lt;/sub&gt;</th>
</tr>
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<tbody>
<tr>
<td>pHK17</td>
<td>300 240</td>
<td>227 105</td>
<td>105 43</td>
<td>73</td>
<td>24</td>
</tr>
<tr>
<td>QpH1</td>
<td>300 110</td>
<td>106 9</td>
<td>9 91</td>
<td>91</td>
<td>63</td>
</tr>
<tr>
<td>QpRS</td>
<td>296 82</td>
<td>112 9</td>
<td>9 89</td>
<td>89</td>
<td>62</td>
</tr>
</tbody>
</table>

* a. E. coli HB101 transduced with C. burnetii QpH1 (NM) or QpRS (PRS) plasmids via the pHK17 cosmid vector. Shown are the mean values of two independent experiments, which produced very similar results. Plates were incubated for 24 h at 37°C, and colonies were enumerated. Plates with 300 or more colonies were considered to exhibit 100% growth.

b. CIP<sup>+</sup> and CIP<sup>−</sup> growth in broth with and without ciprofloxacin (0.006 μg/ml, 18 h, 37°C, 200 rpm), respectively; KAN<sup>+</sup> and KAN<sup>−</sup>, growth on plates with and without kanamycin (50 μg/ml).

c. Ciprofloxacin-induced plasmid loss: [(CIP<sup>−</sup> KAN<sup>−</sup>) – (CIP<sup>−</sup> KAN<sup>+</sup>)].

d. Spontaneous plasmid loss: [(CIP<sup>−</sup> KAN<sup>−</sup>) – (CIP<sup>−</sup> KAN<sup>−</sup>)].

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**TABLE 2. Disk diffusion antibiotic susceptibility assays of E. coli HB101 transductants containing C. burnetii QpH1 (NM) or QpRS (PRS) plasmids**

<table>
<thead>
<tr>
<th>Antibiotic(s)</th>
<th>Concentration in disk</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pHK17</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>5 μg</td>
<td>4/4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 μg</td>
<td>9/10</td>
</tr>
<tr>
<td>Difloxacin</td>
<td>5 μg</td>
<td>10/10</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>5 μg</td>
<td>10/10</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>5 μg</td>
<td>10/10</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>5 μg</td>
<td>10/11</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1 μg</td>
<td>4/5</td>
</tr>
<tr>
<td>Difloxacin</td>
<td>1 μg</td>
<td>3/4</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>1 μg</td>
<td>6/7</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1 μg</td>
<td>6/9</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>1 μg</td>
<td>5/5</td>
</tr>
<tr>
<td>Rifampin</td>
<td>5 μg</td>
<td>1/1</td>
</tr>
<tr>
<td>Chlorotetracycline</td>
<td>30 μg</td>
<td>4/4</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>30 μg</td>
<td>6/6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 μg</td>
<td>4/4</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>2 μg</td>
<td>5/5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 μg</td>
<td>8/8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 μg</td>
<td>7/8</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 μg</td>
<td>6/16</td>
</tr>
<tr>
<td>Polymixin B</td>
<td>10 U</td>
<td>2/2</td>
</tr>
<tr>
<td>Nalidixic acid-polymixin B</td>
<td>5 μg/10 U</td>
<td>4/ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nalidixic acid-rifampin</td>
<td>5/5 μg</td>
<td>5/ND</td>
</tr>
<tr>
<td>Rifampin-polymixin B</td>
<td>5/5 μg/10 U</td>
<td>2/ND</td>
</tr>
<tr>
<td>Rifampin-ciprofloxacin</td>
<td>5/5 μg/10</td>
<td>8/7</td>
</tr>
<tr>
<td>Doxycycline-rifampin</td>
<td>30/5 μg</td>
<td>5/4</td>
</tr>
<tr>
<td>Doxycycline-ciprofloxacin</td>
<td>30/5 μg</td>
<td>7/7</td>
</tr>
</tbody>
</table>

* a. E. coli HB101 transduced with C. burnetii QpH1 (NM) or QpRS (PRS) plasmids via pHK17 cosm, vector.

b. Treated with 0.006 μg of ciprofloxacin per liter in LB broth to deplete plasmids (27) (see Table 1) and grown at 37°C for 18 h with vigorous aeration (200 rpm).

c. ND, not determined.
The roles that *C. burnetii* plasmids may play in virulence, disease syndrome etiology, or antibiotic susceptibility are unknown. The many *C. burnetii* isolates can be categorized into one of three general groups (strains) based upon plasmid character ([13, 23]). These groups include isolates that (i) possess QpH1-type (NM) plasmids, (ii) possess QpRS-type (PRS) plasmids, or (iii) lack free plasmid DNA (S Q217). The NM, S Q217, and PRS isolates used in our investigations were representative of the three major genetic groups of *C. burnetii*.

The basis for the selective toxicity of the tetracyclines—bacterial versus mammalian cells—is permeability: tetracyclines do not normally accumulate within mammalian cells (1, 4, 7, 10, 16). In our studies, we found that L929 mouse fibroblasts persistently infected with different isolates of *C. burnetii* differentially accumulated [3H]tetracycline (Fig. 1). Cells infected with the NM isolate, the most susceptible to tetracycline antibiotics (30–32), accumulated significantly more tetracycline than did cells infected with the S or PRS isolate. It is possible that NM *C. burnetii* infection may result in host cell membrane modification affecting drug uptake, as was also observed in L929 cells recently infected with these isolates. Effects of pH on antibiotic accumulation may be important, since *C. burnetii* proliferates within acid phagolysosomes (2) and has been shown to be metabolically active at pH 4.5 but inactive at pH 7.5 (11). However, in our studies, the *C. burnetii* isolates themselves, purified from infected L929 cells, did not show differential [3H]tetracycline accumulation at either pH 4.5 or 7.5 (Fig. 4 and 5). Collectively, these results suggest that *C. burnetii* isolates that demonstrate decreased susceptibility to tetracycline-class antibiotics may rely upon or perhaps exploit a host cell permeability barrier to avoid exposure to effective concentrations of these drugs. These results lend support to our previous observations that the NM isolate is much more susceptible to tetracycline-class antibiotics than either the S Q217 (30) or the PRS (32) isolate. These data also agree with the previous findings that all *C. burnetii* isolates are more susceptible to antibiotics during recent infection than when involved in long-term persistent infection (30, 32). From these experiments, it appears that the host cell serves as a barrier to tetracyclines for S and PRS *C. burnetii* isolates; whether this phenomenon is mediated by these isolates is not known.

Bacterial resistance to the quinolone-naphthylidine group of antibiotics has been shown to be a result of decreased accumulation and increased active efflux or alteration of the DNA gyrase α-subunit target (5, 12, 25). Quinolones and fluoroquinolones are known to accumulate to effective levels within eukaryotic cells, including neutrophils and macrophages (8, 9). Our results indicate that L929 mouse fibroblast cells independently infected with the NM, S Q217, or PRS isolate exhibit no difference in accumulation of [3H]norfloxacin, a representative fluoroquinolone antibiotic (Fig. 2). However, the different *C. burnetii* isolates did exhibit differential norfloxacin accumulation (Fig. 6 through 9); the accumulation pattern observed correlates with isolate susceptibility to the fluoroquinolones (NM > S Q217 >> PRS [30–32]). These results suggest that isolate, not host cell, permeability accounts for the differential fluoroquinolone resistances of these organisms.

In an attempt to discern whether *C. burnetii* QpH1 and QpRS plasmids are involved in antibiotic resistance, we employed *E. coli* HB101 cells transduced with these plasmids to determine whether their presence altered antibiotic susceptibility. Mallavia and Samuel (13) have previously shown that *C. burnetii* plasmids are efficiently expressed in *E. coli* HB101 transductants. Kirby-Bauer-type disk diffusion assays were used in these determinations (3). When exposed to a variety of antibiotics, including several drug combinations, the pHK17::QpH1 (NM) and pHK17::QpRS (PRS) *E. coli* transductants exhibited nearly identical antibiotic susceptibilities (Table 2), which were equivalent to those of the pHK17-transduced *E. coli* HB101 organism that lacked a *C. burnetii* plasmid (control). These results indicate that, as expressed in *E. coli*, neither the QpH1 nor QpRS *C. burnetii* plasmid mediates antibiotic resistance directly. For the quinolones, at least, this conclusion is reasonable in light of the fact that plasmids have not been shown to mediate resistance to these drugs (6, 25).

The NM, S Q217, and PRS isolates of *C. burnetii* possess distinct plasmids (13, 22), have very different antibiotic susceptibilities (30, 32), and are suspected to cause different clinical syndromes of Q fever (23). As obligate intracellular parasites, these organisms present unique problems concerning their control with antibiotics; effective drugs must be able to penetrate into and accumulate within infected-cell acidic phagolysosomes in active forms (14). Collectively, our observations suggest that differential susceptibilities of the *C. burnetii* isolates to antibiotics are a result of at least two independent mechanisms: differential infected host cell permeability to the tetracycline-class drugs (NM > S Q217 or PRS) and dissimilar permeabilities of the isolates themselves to fluoroquinolone antibiotics (PRS << NM or S Q217). The mechanism responsible for resistance to rifampin observed in the PRS isolate (32) is unknown. No correlation between *C. burnetii* plasmid type and antibiotic susceptibility was found. The conclusion that *C. burnetii* plasmids are not responsible for differences in antibiotic susceptibilities between the NM and PRS isolates is reasonable in light of the broad differences in antibiotic susceptibilities exhibited by the PRS isolate (32).

The results of these studies indicate that the mechanisms responsible for differential antibiotic susceptibilities among the NM, S Q217, and PRS *C. burnetii* isolates are based, at least in part, on permeabilities of the cells to antibiotics. Host cell permeability appears to be involved in *C. burnetii* isolate susceptibility to tetracycline-class antibiotics; the impermeability of the PRS isolate to fluoroquinolones correlates with its resistance to these antibiotics (32). It is clear that much more information concerning the biological characteristics of each *C. burnetii* isolate, as well as the possible roles of their plasmids in pathogenesis, is needed to further understand diversity among *C. burnetii* isolates.

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We thank L. P. Mallavia for providing the *E. coli* HB101 transductants. In addition, we are grateful to William C. Buss and Linus L. Shen, who generously provided the radioactive tetracycline and norfloxacin, respectively, and to Mark Hamden-Smith, who conducted the nuclear magnetic resonance analysis of antibiotics. We also express sincere gratitude to Miriam J. Roman for her excellent technical contributions.

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