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In Vitro Susceptibility of *Coxiella burnetii* to Linezolid in Comparison with Its Susceptibilities to Quinolones, Doxycycline, and Clarithromycin

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The in vitro susceptibility to linezolid shown by nine Greek isolates of *Coxiella burnetii* derived from patients with acute Q fever was investigated. MICs of linezolid were compared with those of pefloxacin, ciprofloxacin, ofloxacin, trovafloxacin, doxycycline, and clarithromycin using the shell vial assay. MICs of linezolid and clarithromycin ranged from 2 to 4 μg/ml; those of doxycycline, trovafloxacin, and ofloxacin ranged from 1 to 2 μg/ml; those of pefloxacin ranged from 1 to 4 μg/ml; and those of ciprofloxacin ranged from 4 to 8 μg/ml. Linezolid was effective in controlling intracellular parasites in cultures of Vero cells infected by *C. burnetii*. No bactericidal activity by linezolid was obtained against *C. burnetii* at 8 μg/ml.

Coxiella burnetii 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. However, the chronic form is a severe disease in which endocarditis predominates (14). Acute *C. burnetii* infection responds easily to antibiotic therapy, while chronic infection is very hard to cure (9, 16, 20).

Linezolid is the first member of a new class of antimicrobial agents, the oxazolidinones. They exhibit a unique mechanism of protein synthesis inhibition and generally display bacteriostatic activity against many important human pathogens, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* (1, 3, 6, 18). Anaerobes such as *Clostridium* spp., *Peptostreptococcus* spp., and *Prevotella* spp. are also susceptible to linezolid. Although linezolid is bacteriostatic against most susceptible organisms, it displays bactericidal activity against some strains of pneumococci, *Bacteroides fragilis*, and *Clostridium perfringens* (1, 21).

The efficacy of linezolid against the acute or chronic form of Q fever infection has yet to be established. In this paper, we report linezolid's activity against the Nine-Mile and Q212 reference strains, as well as against nine Greek isolates of *C. burnetii*. The Greek *C. burnetii* strains were isolated in our laboratory during previous studies (1990 to 2000) from patients with acute Q fever infection (17, 20). We also compared the bacteriostatic effect of linezolid with that of six other antibiotic compounds on the same strains.

A stock solution of linezolid (20 mg/ml; kindly provided by Pharmacia and Upjohn U.S.A.) was prepared using methanol. Pefloxacin (400 mg/5 ml; Rhone Poulenc S.A., Coubevouie, France), ciprofloxacin (100 mg/50 ml; Bayer AG, Leverkuzen, Germany), and ofloxacin (220 mg/100 ml; Hoechst AG, Frankfurt am Main, Germany) for injection were also used in this study. Trovafloxacin (20 mg/ml; kindly provided by Pfizer Inc., New York, N.Y.) was prepared with sterile distilled water. For doxycycline (Pfizer Inc.) and clarithromycin (Abbott Laboratories, Chicago, Ill.), a stock solution at 6 mg/ml was prepared using methanol.

All strains were grown in Vero cells as previously described (4, 15, 17). The bacteriostatic and bactericidal effects on *C. burnetii* were tested by the shell vial assay (4, 7, 15, 19, 22). For this purpose, the concentration of the inoculum that was previously determined to infect 30 to 50% of Vero cells was used to infect the shell vials for the antibiotic challenge (15). 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 30 to 50% infected cells); I for intermediate susceptibility (decreased growth or under 10% infected cells); and S for susceptibility, the absence of infected cells, or the presence of isolated bacteria (no growth) (15).

The bactericidal activity of linezolid was assessed by the quantitative method described by Maurin and Raoult (12). Vero cells were persistently infected with the Nine-Mile and Q212 *C. burnetii* strains for 7 months. In the *C. burnetii* cultures described above, linezolid was added to the culture medium in different concentrations ranging between 1 and 8 μg/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. Infected cells were then lysed, and 10-fold dilutions of the lysate were recultured in Vero cells for 6 days. The presence of viable organisms was identified by immunofluorescence as infecting units per milliliter of culture medium. The bactericidal assay mixtures were made in duplicates and were

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TABLE 1. Susceptibilities of *C. burnetii* isolates to seven antibiotics

Isolate	MIC (μg/ml) of:								
	Pefloxacin (3.8)[a]	Ofloxacin (3)	Ciprofloxacin (1.6)	Trovafloxacin (2.2)	Clarithromycin (4)	Doxycycline (4.4)	Linezolid (13)		
							R[b]	I	S
Nine-Mile	1	1	4	1	2	1	0.5	1	2
Q212	4	2	8	2	4	2	1	2	4
CP1	4	2	8	2	4	2	1	2	4
CP2	1	1	4	1	2	1	0.5	1	2
CP3	1	1	4	1	2	1	0.5	1	2
CP4	2	1	4	1	2	2	0.5	1	2
CP5	1	1	4	1	2	1	0.5	1	2
CP6	1	1	4	1	2	1	0.5	1	2
CP7	1	1	4	1	2	1	0.5	1	2
CP8	1	1	4	1	2	1	0.5	1	2
CP9	1	1	4	1	2	1	0.5	1	2

[a] Values given in parentheses next to names of antibiotics represent C_{max} in micrograms per milliliter.
[b] R, resistant; I, intermediate; S, susceptible.

repeated three times. 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, 11, 12).

Linezolid showed bacteriostatic activity toward *C. burnetii*. Complete bacterial growth inhibition was obtained with an antibiotic concentration of 2 μg/ml with the Nine-Mile strain and with eight Greek isolates. Inhibition was also obtained with an antibiotic concentration of 4 μg/ml in tests with the Q212 strain and the last Greek isolate (Table 1). No bactericidal activity was demonstrated at 8 μg of linezolid/ml. The MICs of all the antibiotics tested are summarized in Table 1.

At the concentrations tested, trovafloxacin, ofloxacin, and doxycycline showed improved bacteriostatic in vitro activity against the Greek *C. burnetii* isolates as well as the tested reference strains. However, pefloxacin, linezolid, and clarithromycin MICs for the same isolates were higher. Ciprofloxacin presented the highest MIC for all tested strains.

Two major problems are associated with the evaluation of the antibiotic treatment of acute Q fever. The acute form is usually a self-limited disease and is mostly retrospectively diagnosed, and in the chronic form, an evaluation of the success of therapy requires prolonged follow-up due to late relapses (9–11, 13–16). Knowledge of various features of acute and chronic disease, such as who will develop chronic disease, remains incomplete. Additionally, reports about the course of untreated infection or the course in those treated with ineffective antibiotics are also missing.

On the other hand, the experimental evaluation of antibiotic therapy is problematic because *C. burnetii* is a strictly intracellular pathogen and because no successful animal model of chronic Q fever has been described so far (8, 15). In cases of acute Q fever, a bacteriostatic effect is sufficient for enabling recovery, whereas in cases of chronic Q fever, a bacteriostatic regimen is not curative (15).

Tetracycline has been the mainstay drug of therapy for endocarditis (14, 16). However, recovery of viable *C. burnetii* from valve tissue after 4 years of therapy with doxycycline has been reported (14). The use of cotrimoxazole alone has failed to cure Q fever endocarditis (14, 15). Combinations of rifampin with either doxycycline or cotrimoxazole have been used in treating Q fever endocarditis, with apparent success (15). Clinical data on the efficacies of macrolides and quinolones are lacking (10).

In our study, linezolid showed bacteriostatic activities (MICs, 2 to 4 μg/ml) in tests with *C. burnetii* strains that were the same as those of clarithromycin and lower than those of ciprofloxacin. The bacteriostatic activity of pefloxacin was also comparable to those of linezolid. Trovafloxacin, ofloxacin, and doxycycline showed improved bacteriostatic in vitro activity compared to linezolid.

The MICs and minimum bactericidal concentrations have been the major parameters to quantify the activity of an antimicrobial against the infecting pathogen. However, in antibiotics exhibiting concentration-dependent killing, such as fluoroquinolones, the ratio of concentration to MIC predicts better bacterial killing of extracellular microorganisms and presumably clinical efficacy. In the case of time-dependent antibiotics such as penicillins, macrolides, and clindamycin, the ratio of time to MIC predicts efficacy (2). To our knowledge, parameters such as the maximum concentration of drug in serum (C_{max}), C_{max}/MIC, or $T >$ MIC are not appropriate for prediction of efficacy of antibiotics against intracellularly sited microorganisms.

In our study, the determination of the activities of linezolid against *C. burnetii* strains showed that, at a concentration of 8 μg/ml, it was not bactericidal to either tested strain. This fact, along with the potential reversible myelosuppression recently presented in rare cases in the literature, should be cautiously taken into account before administration in the cases of Q fever endocarditis where long-term treatment is indicated (5).

In conclusion, our results indicate that linezolid manifests bacteriostatic in vitro activities against *C. burnetii* bacteria. Careful clinical studies are now required to evaluate it for the treatment of acute Q fever infection.

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REFERENCES

1. **Clemett, D., and A. Markham.** 2000. Linezolid. Drugs **59:**815–827.
2. **Craig, W. A.** 1995. Interrelationship between pharmacokinetics and pharmacodynamics in determining dosage regimens for broad-spectrum cephalo-

sporins. Diagn. Microbiol. Infect. Dis. **22:**89–96.
3. **Diekema, D. I., and R. N. Jones.** 2000. Oxazolidinones: a review. Drugs **59:**7–16.
4. **Gikas, A., I. Spyridaki, A. Psaroulaki, D. Kofterithis, and Y. Tselentis.** 1998. In vitro susceptibility of *Coxiella burnetii* to trovafloxacin in comparison with susceptibilities to pefloxacin, ciprofloxacin, ofloxacin, doxycycline, and clarithromycin. Antimicrob. Agents Chemother. **42:**2747–2748.
5. **Green, S. L., J. C. Maddox, and E. D. Huttenbach.** 2001. Linezolid and reversible myelosuppression. JAMA **285:**1291.
6. **Hamel, J. C., D. Stapert, J. K. Moerman, and C. W. Ford.** 2000. Linezolid, critical characteristics. Infection **28:**60–64.
7. **Jabarit-Aldighieri, N., H. Torres, and D. Raoult.** 1992. Susceptibility of *Rickettsia conori*, *R. rickettsii*, and *Coxiella burnetii* to PD 127,391, PD 131,628, pefloxacin, ofloxacin, and ciprofloxacin. Antimicrob. Agents Chemother. **36:**2529–2532.
8. **Keren, G., A. Keysary, R. Goldwasser, and E. Rubinstein.** 1994. The inhibitory effect of fluoroquinolones on *Coxiella burnetii* growth in vitro systems. J. Antimicrob. Chemother. **33:**1253–1255.
9. **Levy, P. Y., M. Drancourt, J. Etienne, J. C. Auvergnat, J. Beytout, J. M. Sainty, F. Goldstein, and D. Raoult.** 1991. Comparison of different antibiotic regimens for therapy of 32 cases of Q fever endocarditis. Antimicrob. Agents Chemother. **35:**533–537.
10. **Maurin, M., and D. Raoult.** 1996. Optimum treatment of intracellular infection. Drugs **52:**45–49.
11. **Maurin, M., A. M. Benoliel, P. Bongrand, and D. Raoult.** 1992. Phagolysosomal alkalinization and the bactericidal effect of antibiotics: the *Coxiella burnetii* paradigm. J. Infect. Dis. **166:**1097–1102.
12. **Maurin, M., and D. Raoult.** 1993. In vitro susceptibilities of spotted fever group rickettsiae and *Coxiella burnetii* to clarithromycin. Antimicrob. Agents Chemother. **37:**2633–2637.
13. **Raoult, D.** 1991. Antibiotic treatment of rickettsiosis, recent advances and current concepts. Eur. J. Epidemiol. **7:**276–281.
14. **Raoult, D., and T. Marrie.** 1995. Q fever. Clin. Infect. Dis. **20:**489–496.
15. **Raoult, D., H. Torres, and M. Drancourt.** 1991. Shell-vial assay: evaluation of a new technique for determining antibiotic susceptibility, tested in 13 isolates of *Coxiella burnetii*. Antimicrob. Agents Chemother. **35:**2070–2077.
16. **Raoult, D.** 1993. Treatment of Q fever. Antimicrob. Agents Chemother. **37:**1733–1736.
17. **Spyridaki, I., A. Gikas, D. Kofteridis, A. Psaroulaki, and Y. Tselentis.** 1998. Q fever in the Greek island of Crete: detection, isolation, and molecular identification of eight strains of *Coxiella burnetii* from clinical samples. J. Clin. Microbiol. **36:**2063–2067.
18. **Swaney, S. M., H. Aoki, M. C. Ganoza, and D. L. Shinabarger.** 1998. The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. Antimicrob. Agents Chemother. **42:**3251–3255.
19. **Torres, H., and D. Raoult.** 1993. In vitro activities of ceftriaxone and fusidic acid against 13 isolates of *Coxiella burnetii*, determined using the shell vial assay. Antimicrob. Agents Chemother. **37:**491–494.
20. **Tselentis, Y., A. Gikas, D. Kodteridis, E. Kyriakakis, N. Lydataki, D. Bouros, and N. Tsaparas.** 1995. Q fever in the Greek island of Crete: epidemiologic, clinical, and therapeutic data from 98 cases. Clin. Infect. Dis. **20:**1311–1316.
21. **Wise, R., J. M. Andrews, F. J. Boswell, and J. P. Ashby.** 1998. The in vitro activity of linezolid (U-100766) and tentative breakpoints. J. Antimicrob. Chemother. **42:**721–728.
22. **Yeaman, M. R., L. A. Mitscher, and O. G. Baca.** 1987. In vitro susceptibility of *Coxiella burnetii* to antibiotics, including several quinolones. Antimicrob. Agents Chemother. **31:**1079–1084.