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Susceptibility of the Lyme Disease Spirochete to Seven Antimicrobial Agents

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The antimicrobial susceptibility of five Lyme disease spirochete strains (two human and three tick isolates) was determined. A macrodilution broth technique was used to determine on three separate test occasions the minimal inhibitory concentrations (MICs) of seven antibiotics. The Lyme disease spirochete was most susceptible to erythromycin with a MIC of ≤ 0.06 μg/ml. The spirochete was also found to be susceptible to minocycline, ampicillin, doxycycline, and tetracycline-HCL with respective mean MICs of ≤ 0.13, ≤ 0.25, ≤ 0.63, and ≤ 0.79 μg/ml. The spirochete was moderately susceptible to penicillin G with a mean MIC of 0.93 μg/ml. All strains were resistant to rifampin at the highest concentration tested (16.0 μg/ml).

Lyme disease is a recently described human disorder characterized initially by erythema chronicum migrans (ECM) that is often followed by cardiac, neurologic, and/or arthritic complications. ECM was initially described in Europe where treatment with penicillin was effective [1-3]. Subsequently, it has been reported in the United States that ECM and its associated sequelae may be prevented or ameliorated by treatment with antimicrobials such as penicillin and tetracycline [4,5]. In 1983, Steere et al. [6] found that tetracycline was superior to penicillin in preventing these sequelae. Until recently, *in vitro* evaluation of these clinical observations was not possible because the etiologic agent for this disease had not been recovered.

A spirochete referred to here as the Lyme disease spirochete (LDS) has now been isolated [7-12]. To compare the reported clinical efficacy of antimicrobials with *in vitro* results and to select additional antimicrobials of potential therapeutic benefit, we tested the efficacy of seven antimicrobials in an *in vitro* assay against five LDS strains (Table 1). To ensure the detection of any possible difference in strain-to-strain antimicrobial susceptibility, we selected strains to represent different geographical locations and various hosts because of: *(a)* the occurrence of Lyme disease or ECM in various areas in the United States and other countries [13-18]; *(b)* the isolation of LDS from *Ixodes dammini* ticks in the United States [10-12] and *I. ricinis* ticks from Switzerland [7]; and *(c)* the isolation of LDS from blood, cerebrospinal fluid, and ECM lesions of Lyme disease patients [8,12]. We report here a brief review of these findings, which are reported in greater detail elsewhere [19].

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TABLE 1
Suppliers and Sources of the Lyme Disease Spirochete Strains Tested

Strain Identification	Supplier	Source
FIS 001	A.G. Barbour[a] (B31)[b]	*Ixodes dammini* tick from Shelter Island, NY
FIS 004	A.C. Steere[c] (243)	*Ixodes dammini* tick from Great Island, MA
FIS 005	A.C. Steere (245)	Human blood from a Lyme disease patient in CT
FIS 008	A.G. Barbour	*Ixodes ricinus* tick from Switzerland
FIS 033	J.L. Benach[d]	Human blood from a Lyme disease patient in NY

[a]Rocky Mountain Laboratories, National Institute of Health, Hamilton, Montana
[b]Supplier's strain identification is given in parentheses.
[c]Yale University School of Medicine, New Haven, Connecticut
[d]New York State Department of Health and State University of New York at Stony Brook, Stony Brook, New York

MATERIALS AND METHODS

For our study, Barbour, Stoenner, Kelly (BSK) medium [7; Barbour AG: Personal communication] was used and prepared as previously described [11]. Since we had not used BSK medium previously for *in vitro* antimicrobial susceptibility testing, we first determined its effects on antimicrobial activity by testing simultaneously two control organisms, *Staphylococcus aureus* ATCC 29213 and *Streptococcus faecalis* ATCC 29212, in BSK and cation supplemented Mueller-Hinton broth media containing each antimicrobial to be used in our study (Table 2). A macrodilution broth technique was used for both control organisms and LDS to determine the minimal inhibitory concentrations (MICs) of seven antimicrobials on three separate occasions. The antimicrobials tested and their respective twofold dilu-

TABLE 2
Antimicrobials, Susceptibility Criteria, and Concentrations to Which the Lyme Disease Spirochete Was Tested

Antimicrobic	Susceptibility Criteria[a,b]			Concentrations[b,c] Tested
	Susceptible	Moderately Susceptible	Resistant	
Ampicillin	≤1.0	2.0–16.0	>16.0	0.25–16.0
Penicillin G	≤0.12	0.25–16.0	>16.0	0.06–4.0
Erythromycin	≤0.5	1.0–4.0	> 4.0	0.06–4.0
Doxycycline	≤1.0	2.0–8.0	> 8.0	0.25–16.0
Minocycline	≤1.0	2.0–8.0	> 8.0	0.12–8.0
Tetracycline-HCl	≤1.0	2.0–8.0	> 8.0	0.25–16.0
Rifampin	≤2.0	4.0	> 4.0	0.25–16.0

[a]As suggested by the National Committee for Clinical Laboratory Standards [20]
[b]In μg/ml
[c]In serial twofold dilutions

tion ranges are listed in Table 2. Stock solutions (1,056 μg/ml) of each antimicrobial, except rifampin, were prepared, filter-sterilized (pore size of 0.2 μ), and stored at −70°C in 5 ml aliquots until ready to use. Rifampin at 528 μg/ml was made fresh before each test and was not filtered. A series of twofold working dilutions was prepared on test day for each of the seven antimicrobials. The dilutions were prepared by the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [20]. One-tenth ml of the appropriate working dilution was added to culture tubes (13 × 100, plastic, No. 2027, Falcon, Oxnard, California) containing 6.4 ml of BSK medium. These tubes and growth control tubes (BSK without antimicrobials) were inoculated to a final density of 10^5 cells per ml with a five-day-old culture of the appropriate LDS strain in BSK medium containing 10^7 actively growing cells per ml.

In testing the effect of BSK medium on antimicrobial activity and for quality control of the antimicrobials during each of the three test runs, *S. aureus* and *S. faecalis,* grown on heart infusion agar supplemented with 5 percent defibrinated rabbit blood for 18–20 hours at 33°C in air, were suspended in Mueller-Hinton broth (10^7 cells per ml) and similarly inoculated into separate sets of tubes containing the appropriately diluted antimicrobals. After 120 hours' incubation in air at 33°C the numbers of motile LDS in test cultures were determined by darkfield microscopy and a Petroff-Hausser bacteria counter. Three counts were taken and averaged for each culture. The least concentration of antimicrobial that had $\leq 10^5$ motile LDS per ml with no noticeable cell sediment was considered the MIC. After 20 hours' incubation in air at 33°C, MICs were determined for *S. aureus* and *S. faecalis.* The least concentration of antimicrobial that completely inhibited growth (no turbidity and cell sediment) as determined by the unaided eye was considered the MIC.

RESULTS

In the MIC comparison study our results on media influence showed that MICs for the evaluated antimicrobials were similar and that they were within acceptable tolerances (±1 doubling dilution) of the expected values. Guided by these results and the susceptibility standards recommended by the NCCLS [20] for the antimicrobials tested (Table 2), we found that all the strains of LDS tested were either susceptible or moderately susceptible to all of the antimicrobials evaluated except rifampin (Table 3). Of the beta-lactams tested, ampicillin was more active than

TABLE 3
MICs of Seven Antimicrobials and Their Susceptibility Interpretation for Five Strains of Lyme Disease Spirochete

Antimicrobic	Class	MICs (μg/ml) Mean[a]	MICs (μg/ml) Range	Susceptibility Interpretation
Ampicillin	Beta-lactam	≤0.25	0	Susceptible
Penicillin G	Beta-lactam	0.93	0.25–2.0	Moderately susceptible
Erythromycin	Macrolide	≤0.06	0	Susceptible
Doxycycline	Tetracycline	≤0.63	≤0.25–2.0	Susceptible
Minocycline	Tetracycline	≤0.13	≤0.12–0.25	Susceptible
Tetracycline-HCl	Tetracycline	≤0.79	≤0.25–2.0	Susceptible
Rifampin	Rifamycin	>16.0	0	Resistant

[a]Geometric mean of 15 determinations; three determinations were made for each of the five strains of Lyme disease spirochete tested.

penicillin G; of the tetracyclines, minocycline was more active than either tetracycline-HCl or doxycycline; and the macrolide erythromycin had the lowest MIC of all the antimicrobials tested.

DISCUSSION

The fact that penicillin G and tetracycline-HCl were active against LDS *in vitro* supports the observations of Steere et al. [5,6] that the therapeutic use of these drugs reduces the duration of ECM and either prevents or ameliorates neurologic, cardiac, or arthritic sequelae. Our data also show that other antimicrobials were more effective than these drugs in inhibiting the growth of LDS, suggesting that differences in efficacy *in vivo* might be expected from different penicillin or tetracycline preparations.

The reasons for the contrasting results between the high *in vitro* activity of erythromycin and the poorer efficacy *in vivo* when compared to penicillin and tetracycline are unclear. Such discrepancies between high *in vitro* activity and lesser clinical efficacy may be seen with other organisms (e.g., *Legionella*) [21]. The phenomenon emphasizes that our results provide a guide only to antimicrobials that may be effective *in vivo*.

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