JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2003, p. 1791–1793 Vol. 41, No. 4
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.4.1791–1793.2003
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In Vitro Susceptibility Testing of Four Antibiotics against *Borrelia burgdorferi*: a Comparison of Results for the Three Genospecies *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia burgdorferi* Sensu Stricto

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Received 1 October 2002/Returned for modification 6 November 2002/Accepted 20 December 2002

MICs and minimal bactericidal concentrations (MBCs) were evaluated for the four antibiotics azithromycin, amoxicillin, ceftriaxone, and doxycycline against the three main genospecies of *Borrelia burgdorferi* sensu lato. In MBC testing, statistically significant differences between the genospecies could be found in 7 out of 12 comparative evaluations ($P < 0.05$).

Lyme borreliosis is a multisystemic disease caused by the spirochete *Borrelia burgdorferi*, which was discovered in 1981 (5). It is transmitted by tick vectors of the genus *Ixodes* (13, 15).

Today, several different genospecies of the spirochete *Borrelia* are known. Three of them are of a special clinical relevance: *B. burgdorferi* sensu stricto is the only one endemic in North America, whereas *B. afzelii* and *B. garinii* are predominant in Europe (1, 4, 14, 16, 17).

Many in vitro (3, 6–8, 10, 12) and in vivo (11) studies have been performed to find an optimal treatment for Lyme disease; what form such a treatment would take is still not quite clear (12). On the other hand, only few data (3, 6–8, 10, 12) are available about possible differences in the antibiotic sensitivities of the three main genospecies, and the results obtained thus far are quite controversial.

Considering the geographic pattern of distribution mentioned above, we found it necessary to look for differences in the antibiotic responses of *B. garinii*, *B. afzelii*, and *B. burgdorferi* sensu stricto. The results could contribute to answering the question of whether Lyme disease should be treated differently in America and Europe.

The four antibiotics used for this study were azithromycin (Pfizer, New York, N.Y.), amoxicillin, ceftriaxone, and doxycycline (Sigma, Munich, Germany).

All strains of *B. burgdorferi* except strain B31 (imported from the United States) were low-passage isolates (not more than 10 passages). The isolates were obtained from either ticks or skin lesions at the Department of Dermatology, Ludwig-Maximilians University, Munich, Germany, or kindly provided by A. Vogt, University Clinic of Freiburg, Freiburg, Germany. Identification of the strains was performed by PCR typing as previously described (1, 16, 17). We chose a total of 24 strains (8 strains of each genospecies) (Table 1).

The antibiotics were dissolved in BSK-H medium (Sigma-Aldrich, Deisenhofen, Germany) supplemented with 6% rabbit serum and were stored in 2.5-ml test tubes. The final concentration ranges were 0.125 to 16 μg/ml for doxycycline and 0.002 to 0.5 μg/ml for the other tested antimicrobial substances. They were chosen according to the expected MICs and minimal bactericidal concentrations (MBCs) described in the literature (3, 6–8, 10, 12).

The MIC was defined as the lowest concentration of antibiotics at which the number of countable cells after 72 h of incubation at 33°C did not exceed the basis concentration. To determine the MIC, 50 μl of BSK-H medium containing *B. burgdorferi* was added to the test tubes described above. Using the method of dark-field microscopy, the basis concentration was adjusted to 10^5 cells/ml. From each tube, 5 μl of BSK-H medium was pipetted on a cover glass. The vital spirochetes in 10 fields of view were counted under the microscope. The concentration of bacteria in the test tubes was determined by using the following formula:

$$\text{number of cells per ml} = \frac{\text{average number of cells per field of view} \times \text{cover glass size}}{\text{field of view size} \times \text{medium per cover glass}}$$

In our study, the technical data were as follows: cover glass size, 400 mm^2; field of view size, 0.159 mm^2.

This testing was done in triplicate for each strain. A test tube containing BSK medium without antibiotics was used as a control. The test tubes were then incubated for 72 h at 33°C. After that period, the cells were counted again.

The MBC was defined as the lowest concentration of antibiotics at which no viable spirochetes could be detected after 2 weeks of subcultivation in BSK-H medium free of antibiotics at 33°C. To determine the MBC, 50 μl of the contents of each of the test tubes which had been used earlier for MIC determination and which contained antibiotics at the MIC or a higher concentration was added to 2.5 ml of BSK-H without antibiotics. The mixtures were incubated for 14 days at 33°C, and then the cells were counted again.

Statistical analysis was done by comparisons of the confidence intervals and by the Kruskal-Wallis H test and the Wilcoxon ranking test. The level of significance was $P < 0.05$.

There were no statistically significant differences between

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TABLE 1. MICs and MBCs of four antimicrobial agents against *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia burgdorferi* sensu stricto after 72 h of incubation at 33°C

Genospecies	Strain (alternative name)	MIC or MBC of:							
		Azithromycin		Amoxicillin		Ceftriaxone		Doxycycline	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. afzelii	A-1	0.015	0.125	0.06	0.5	0.03	0.06	1	16
B. afzelii	A-2	0.008	0.06	0.03	0.5	0.03	0.03	0.5	8
B. afzelii	A-3	0.015	0.03	0.25	0.5	0.03	0.03	0.25	4
B. afzelii	A-4 (PKO)	0.008	0.125	0.25	>0.5	0.03	0.06	0.5	8
B. afzelii	A-5	0.015	0.03	0.03	0.5	0.03	0.03	1	4
B. afzelii	A-6	0.015	0.125	0.03	0.5	0.03	0.06	1	16
B. afzelii	A-7	0.03	0.125	0.06	0.5	0.03	0.06	1	8
B. afzelii	A-8	0.015	0.125	0.25	>0.5	0.03	0.06	2[a]	16
B. garinii	G-1	0.015	0.03	0.03	0.5	0.015	0.06	0.25	16
B. garinii	G-2	0.008	0.015	0.25	0.5	0.03	0.06	1	8
B. garinii	G-3	0.008	0.015	0.06	0.5	0.03	0.06	0.5	4
B. garinii	G-4	0.03	0.03	0.03	0.5	0.03	0.06	0.5	16
B. garinii	G-5	0.008	0.015	0.03	0.5	0.03	0.06	0.5	8
B. garinii	G-6	0.015	0.03	0.06	0.5	0.03	0.06	0.5	4
B. garinii	G-7	0.015	0.03	0.06	0.5	0.03	0.06	1	8
B. garinii	G-8	0.03	0.03	0.03	0.5	0.03	0.06	1	8
B. burgdorferi sensu stricto	S-1 (B31)	0.03	0.25	0.06	0.125	0.03	0.06	0.5	8
B. burgdorferi sensu stricto	S-2	0.015	0.03	0.25	0.25	0.03	0.25	1	16
B. burgdorferi sensu stricto	S-3	0.03	0.125	0.03	0.5	0.015	0.06	0.25	4
B. burgdorferi sensu stricto	S-4	0.008	0.06	0.03	0.5	0.015	0.125	0.5	4
B. burgdorferi sensu stricto	S-5	0.008	0.015	0.125	0.25	0.03	0.125	4[a]	>16
B. burgdorferi sensu stricto	S-6	0.015	0.015	0.015	0.25	0.03	0.06	1	16
B. burgdorferi sensu stricto	S-7	0.008	0.125	0.06	0.5	0.03	0.06	0.5	8
B. burgdorferi sensu stricto	S-8	0.015	0.06	0.25	0.5	0.06	0.25	0.25	4

[a] Marginally susceptible; all other strains were susceptible.

the MICs of the tested antibiotics against *B. afzelii*, *B. garinii*, and *B. burgdorferi* sensu stricto (Table 1).

In 7 out of 12 comparative evaluations ($P < 0.05$), MBCs were significantly different among the three genospecies (Table 1). *B. garinii* seemed to be especially susceptible to azithromycin, while amoxicillin had a significantly greater effect on *B. burgdorferi* sensu stricto compared to the other genospecies. Ceftriaxone had the lowest MBC with *B. afzelii* and increasingly higher MBCs with *B. garinii* and *B. burgdorferi* sensu stricto. Doxycycline did not show any remarkable differences in its effects on the three genospecies.

The results of our study contradict those of Baradaran-Dilmaghani et al., who found no statistically significant differences between the genospecies (3), but partially confirm those of Preac-Mursic et al. (12), who described susceptibilities of *B. garinii* to amoxicillin, doxycycline, cefotaxime, ceftriaxone, azithromycin, and penicillin G that were higher than those of *B. afzelii*. Hunfeld et al., too, found *B. garinii* to be the genospecies most susceptible to macrolides and cephalosporins (9). As in our study, Henneberg and Neubert describe *B. burgdorferi* sensu stricto as being comparatively less susceptible to cephalosporins, but they also found *B. garinii* to be more sensitive than the other genospecies to penicillin (6).

With the exception of doxycycline, the antibotics tested by us showed excellent efficacy against all 24 borrelial strains. Indeed, we found some statistically significant differences between antibiotic MBCs for the three genospecies.

B. burgdorferi sensu stricto, the etiologic agent of North American Lyme disease, showed the highest susceptibility of the three species to amoxicillin. *B. afzelii*, the predominant borrelial species in Central Europe and the causative organism of acrodermatitis chronica atrophicans (2, 17), proved to be especially susceptible to ceftriaxone. *B. garinii*, the most frequently isolated species from cerebrospinal fluid in European neuroborreliosis (14), demonstrated excellent sensitivity to azithromycin. However, these differences in antibiotic sensitivity seem not sufficiently pronounced to be of fundamental clinical relevance.

Beyond these findings, one has to consider that the different pharmacokinetics of the particular antibiotics do not allow a simple transfer of our in vitro results to practical treatment. For instance, the concentration of azithromycin in the cerebrospinal fluid is too low to represent a promising alternative to standard therapeutic regimens of neuroborreliosis such as intravenous infusion of ceftriaxone (9).

All together, the results of our study do not justify specific recommendations concerning antibiotic therapy of Lyme borreliosis for the suspected causative borrelial species.

We thank J. Laude and G. Weishaar (Ludwig-Maximilians University, Munich, Germany) for excellent technical assistance.

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