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In Vitro Susceptibilities of Seven *Leptospira* Species to Traditional and Newer Antibiotics

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Human leptospirosis is generally treated with penicillin or doxycycline. We studied the susceptibilities of 11 serovars (seven species) of *Leptospira* to 14 antibiotics. With the exception of chloramphenicol, all tested agents were at least as potent as penicillin and doxycycline, with the macrolide and ketolide drugs producing the lowest MICs (and minimal bactericidal concentrations).

Leptospirosis is a zoonotic infection with a worldwide distribution that is associated with both endemic disease and epidemics, with the incidence of disease being highest in tropical climates (6). Infection can range in severity from clinically inapparent to life threatening, with an epidemic case fatality rate as high as 15% (5). Limited studies have examined the in vitro and in vivo effects of antibiotics against *Leptospira* and leptospirosis. Two placebo-controlled human trials of intravenous penicillin therapy in severe or icteric disease (4, 14) and a single trial of oral doxycycline therapy in acute febrile illness (7) have found these agents to be effective in decreasing symptoms, including days of fever, and in resolving leptospiruria. In vitro testing is not standardized and is chiefly limited to antibiotics available prior to 1993 (3, 10, 13). In two of the largest published studies, 16 antimicrobial agents (penicillin G and V, ampicillin, piperacillin, apalcillin, cephalothin, cefmetazole, moxalactam, cefoperazone, ceftizoxime, cefotaxime, streptomycin, kanamycin, tobramycin, amikacin, and tetracycline) were tested against five serovars (10) and six drugs (Q-35, norfloxacin, ofloxacin, ciprofloxacin, tosufloxacin, and tetracycline) were tested against five serogroups (13). In a fatal hamster model, ampicillin, piperacillin, mezlocillin, doxycycline, and cefotaxime were shown to prevent death (1). We examined the MICs and minimal bactericidal concentrations (MBCs) of 14 antibiotics against 11 *Leptospira* spp. isolates using a macrodilution broth method.

Isolates of *Leptospira* were obtained from the Veterinary Command Food and Drug Analysis Laboratory, Fort Sam Houston, Tex. These strains originated at the U.S. Department of Agriculture National Veterinary Services Laboratories, Ames, Iowa. Strains tested include *Leptospira biflexa* serovar Patoc (serogroup Semaranga, strain Patoc I), *Leptospira borgpetersenii* serovar Ballum (serogroup Ballum, strain S 102), *L. borgpetersenii* serovar Sejroe (serogroup Sejroe, strain M 84), *Leptospira interrogans* serovar Copenhageni (serogroup Icterohaemorrhagiae, strain M 20), *L. interrogans* serovar Grippotyphosa (serogroup Grippotyphosa, strain Andaman), *L. interrogans* serovar Icterohaemorrhagiae (serogroup Icterohaemorrhagiae, strain RGA), *L. interrogans* serovar Pomona (serogroup Pomona, strain Pomona), *Leptospira kirschneri* serovar Butembo (serogroup Autumnalis, strain Butembo), *Leptospira noguchii* serovar Fortbragg (serogroup Autumnalis, strain Fort Bragg), *Leptospira santarosai* serovar Alexi (serogroup Pyrogenes, strain HS 616), and *Leptospira weilii* serovar Celledoni (serogroup Celledoni, strain Celledoni). Isolates were maintained in continuous culture in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium (Becton Dickinson, Sparks, Md.). Inoculum was produced from cultures grown for 7 days at 30°C, quantified by organism count using a Petroff-Hausser counting chamber under dark-field microscopy.

Amoxicillin, ampicillin, cefotaxime, ceftriaxone, chloramphenicol, doxycycline, erythromycin, penicillin G, and tetracycline were purchased from Sigma-Aldrich (St. Louis, Mo.). Other antibiotics were obtained from their manufacturers (ampicillin/sulbactam and azithromycin, Pfizer, Groton, Conn.; ciprofloxacin and moxifloxacin, Bayer, West Haven, Conn.; telithromycin, Aventis, Bridgewater, N.J.). Stock 1-mg/ml solutions were produced by using the solvents and diluents suggested in NCCLS document M7-A4 (9) or by their manufacturers. Aliquots of stock solutions were stored at −70°C until the day of use.

MIC and MBC testing was performed by using a broth macrodilution technique similar to that previously described (10). Serial twofold dilutions of antibiotics in EMJH medium resulted in final concentrations of 100 to 0.01 μg/ml (penicillin concentrations are in units per milliliter; ampicillin/sulbactam was studied in a 2:1 ratio, as found in the ampicillin/sulbactam combination Unasyn [results recorded are based on ampicillin content]). *Leptospira* inoculum was added to produce a final concentration of 10^6 organisms/ml (final volume, 2 ml), and tubes were incubated at 30°C for 7 days. The lowest concentration without visual growth was recorded as the MIC. After MIC determination, 10 μl was transferred from tubes without visible growth into 2 ml of EMJH medium and incubated for 3 weeks at 30°C. The lowest antibiotic concentration that yielded no growth by visual inspection at 3 weeks was documented as the MBC.

Results are displayed in two groups: traditional antibiotics, including doxycycline, penicillin, and other older antibiotics, in Table 1 and newer antibiotics in Table 2. MICs that suppressed

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TABLE 1. MICs and MBCs of traditional antibiotics for 11 serovars of *Leptospira*

Species and serovar	MIC (MBC)[a]						
	Penicillin	Ampicillin	Amoxicillin	Doxycycline	Tetracycline	Chloramphenicol	Erythromycin
L. biflexa, Patoc	0.20 (50)	0.10 (25)	0.20 (50)	0.78 (25)	0.78 (50)	0.39 (>100)	≤0.01 (0.10)
L. borgpetersenii, Ballum	0.05 (3.13)	0.05 (25)	≤0.01 (6.25)	0.78 (12.5)	0.78 (3.13)	1.56 (3.13)	≤0.01 (≤0.01)
L. borgpetersenii, Sejroe	0.39 (25)	0.20 (6.25)	0.20 (12.5)	0.78 (3.13)	0.78 (3.13)	1.56 (6.25)	≤0.01 (≤0.01)
L. interrogans, Copenhageni	0.39 (25)	0.05 (6.25)	0.05 (1.56)	0.78 (12.5)	1.56 (6.25)	1.56 (25)	≤0.01 (0.20)
L. interrogans, Grippotyphosa	0.39 (12.5)	0.05 (1.56)	0.05 (1.56)	0.78 (3.13)	0.78 (3.13)	1.56 (12.5)	≤0.01 (≤0.01)
L. interrogans, Icterohaemorrhagiae	0.78 (50)	0.20 (25)	0.20 (25)	1.56 (6.25)	1.56 (6.25)	1.56 (12.5)	≤0.01 (0.10)
L. interrogans, Pomona	3.13 (100)	0.10 (25)	0.05 (12.5)	3.13 (50)	3.13 (25)	3.13 (>100)	≤0.01 (0.39)
L. kirschneri, Butembo	0.78 (1.56)	0.10 (0.10)	0.02 (0.05)	0.10 (0.10)	0.39 (0.39)	1.56 (1.56)	≤0.01 (0.02)
L. noguchii, Fortbragg	0.20 (25)	0.05 (3.13)	0.05 (6.25)	1.56 (12.5)	1.56 (6.25)	1.56 (100)	≤0.01 (0.20)
L. santarosai, Alexi	3.13 (50)	0.78 (25)	0.10 (25)	3.13 (12.5)	1.56 (12.5)	3.13 (50)	≤0.01 (0.02)
L. weilii, Celledoni	0.20 (12.5)	0.02 (0.39)	0.02 (0.10)	0.39 (6.25)	0.78 (1.56)	1.56 (1.56)	≤0.01 (≤0.01)
MIC_{90} (MBC_{90})	3.13 (50)	0.20 (25)	0.20 (25)	3.13 (25)	1.56 (25)	3.13 (>100)	≤0.01 (0.20)

[a] Values for penicillin are in units per milliliter; all others are in micrograms per milliliter.

90% of the strains (MIC_{90}s) and MBC_{90}s are included for comparison.

Doxycycline and penicillin are currently the drugs of choice in the treatment of human leptospirosis, based chiefly on the fact that they are the only agents that have been studied in randomized controlled clinical trials (4, 7, 14). Small studies have shown that the newer cephalosporins and other β-lactams, as well as fluoroquinolone antibiotics, all have good in vitro activity against strains of *Leptospira*. Animal treatment models have documented activity of doxycycline, penicillin, ampicillin, chlortetracycline, erythromycin, piperacillin, mezlocillin, moxalactam, cefotaxime, and ciprofloxacin against lethal infection with individual serovars (1, 2).

Our study confirms prior reports of the activity of penicillin, amoxicillin, ampicillin, erythromycin, ciprofloxacin, cefotaxime, and ceftriaxone against multiple previously untested *Leptospira* strains (3, 8, 10, 11, 12, 13). Most previous studies of antimicrobial susceptibility have been limited to the use of only one (3, 12) or two (8) *Leptospira* isolates of interest. Thus, our study expands the data to include a more diverse range of strains and species. The macrolide antibiotics erythromycin and azithromycin produced excellent in vitro activity against all 11 strains studied. The recently approved agent telithromycin, first of the new ketolide antibiotics, showed the best activity of all antibiotics tested. The expanded-spectrum cephalosporins cefotaxime and ceftriaxone and the fluoroquinolones ciprofloxacin and moxifloxacin also appear to have excellent activity against leptospires. Ampicillin and amoxicillin had lower MICs than penicillin or doxycycline for almost all tested strains. Interesting, the addition of a β-lactamase inhibitor greatly enhanced the activity of ampicillin, especially in regard to its MBC. Whether this implies additive activity of the sulbactam or production of β-lactamase by these organisms requires further examination, as this has not been described.

MBC results revealed potentially important differences between many of the newer agents and erythromycin compared to the traditional antibiotics. As expected, the MBC_{90}s of all drugs were higher than noted MIC_{90}s. MBC_{90}s of erythromycin, azithromycin, telithromycin, cefotaxime, ceftriaxone, and the combination of ampicillin with sulbactam all were lower than the MIC_{90}s of penicillin and doxycycline. Only chloramphenicol produced a higher MBC_{90} than the latter two agents.

How these noted in vitro results correlate with in vivo efficacy is not clear. The next step in examining the activity of the promising agents is to test them in an animal model. In vivo activity in animal models could allow selection of agents for human treatment trials.

TABLE 2. MICs and MBCs of newer antibiotics for 11 serovars of *Leptospira*

Species and serovar	MIC (MBC)						
	Ampicillin-sulbactam[a]	Cefotaxime	Ceftriaxone	Azithromycin	Telithromycin	Ciprofloxacin	Moxifloxacin
L. biflexa, Patoc	0.05 (0.10)	0.05 (0.10)	0.10 (0.39)	≤0.01 (0.39)	≤0.01 (0.20)	≤0.01 (1.56)	≤0.01 (3.13)
L. borgpetersenii, Ballum	0.02 (0.05)	≤0.01 (0.02)	0.05 (0.10)	0.02 (0.05)	≤0.01 (≤0.01)	0.10 (12.5)	0.10 (12.5)
L. borgpetersenii, Sejroe	0.05 (0.10)	≤0.01 (≤0.01)	0.02 (0.10)	≤0.01 (≤0.01)	≤0.01 (≤0.01)	0.39 (25)	0.20 (12.5)
L. interrogans, Copenhageni	0.02 (0.10)	≤0.01 (0.05)	0.02 (0.20)	0.05 (0.20)	≤0.01 (≤0.01)	0.20 (0.78)	0.10 (0.20)
L. interrogans, Grippotyphosa	0.02 (0.05)	≤0.01 (0.05)	0.02 (0.10)	≤0.01 (≤0.01)	≤0.01 (≤0.01)	0.05 (0.10)	0.05 (0.10)
L. interrogans, Icterohaemorrhagiae	0.02 (0.05)	≤0.01 (0.10)	≤0.01 (0.78)	≤0.01 (0.39)	≤0.01 (≤0.01)	0.10 (12.5)	0.10 (3.13)
L. interrogans, Pomona	0.02 (0.10)	0.02 (0.05)	0.10 (0.39)	≤0.01 (1.56)	≤0.01 (0.10)	0.10 (12.5)	0.05 (12.5)
L. kirschner, Butembo	0.05 (0.20)	0.05 (0.10)	0.20 (0.20)	≤0.01 (≤0.01)	≤0.01 (≤0.01)	0.20 (0.20)	0.10 (0.10)
L. noguchii, Fortbragg	≤0.01 (0.05)	≤0.01 (≤0.01)	≤0.01 (0.10)	≤0.01 (0.78)	≤0.01 (0.05)	0.10 (12.5)	0.05 (12.5)
L. santarosai, Alexi	0.05 (0.10)	0.10 (0.20)	0.20 (0.39)	≤0.01 (0.39)	≤0.01 (0.10)	0.10 (25)	0.10 (12.5)
L. weilii, Celledoni	0.02 (0.05)	0.05 (0.05)	0.10 (0.10)	≤0.01 (0.02)	≤0.01 (≤0.01)	0.10 (0.20)	0.10 (0.10)
MIC_{90} (MBC_{90})	0.05 (0.10)	0.05 (0.10)	0.20 (0.39)	0.02 (0.78)	≤0.01 (0.10)	0.20 (25)	0.10 (12.5)

[a] Ampicillin-sulbactam was used in a 2:1 mixture; results are reported by ampicillin content.

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