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# Broth Microdilution Susceptibility Testing for *Leptospira* spp.

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**Leptospirosis in humans has traditionally been treated with penicillin or doxycycline. The choice of therapy offered at the time of initial patient presentation is often empirical, as definitive diagnosis can take weeks. Determining the activity of numerous antimicrobial agents against a wide range of *Leptospira* serovars may broaden empirical therapeutic options. Various antimicrobials have been shown to be active against a limited number of serovars in in vitro studies, chiefly by the use of broth macrodilution techniques. We developed a broth microdilution technique using the commercially available growth indicator alamarBlue. MICs produced by this technique were compared to MICs and minimal bactericidal concentrations produced by the traditional broth macrodilution technique. The internal validity of our methods was assessed with 11 runs over numerous days with a single isolate of *Leptospira interrogans* serovar Icterohaemorrhagiae. By either method, the MICs for these internal-validity runs fell within 2 dilutions of each other for more than 90% of antimicrobials. A broader application of these two techniques included 12 serovars (including seven species) of *Leptospira* and six antimicrobials (penicillin G, doxycycline, chloramphenicol, erythromycin, cefotaxime, and ciprofloxacin). Observed reproducibility fell within 2 dilutions for 99% of the duplicate result sets for the MIC microdilution method, compared to 89% for the MIC macrodilution method. The macrodilution method tended to have a higher MIC at which 90% of the isolates were inhibited ($MIC_{90}$) than did the microdilution method, but the $MIC_{90}$s of both methods were within 2 dilutions of each other for all six drugs. The macrodilution and microdilution techniques produced similar results, with microdilution allowing a faster, more streamlined method of producing MIC results.**

Spirochetal bacteria of the genus *Leptospira* are responsible for zoonotic cosmopolitan infections causing both endemic and epidemic disease. Infection may be clinically inapparent or may produce a febrile illness ranging in severity from mild to life threatening. Therapeutic decisions are often empirical, as definitive diagnosis is based on cultures that have a low yield (and require incubation of 4 to 6 weeks) or on the analysis of acute- and convalescent-phase sera. Therefore, treatment is often based on a differential diagnosis that includes leptospirosis, necessitating broad antimicrobial therapy against a number of infectious agents.

Data regarding the in vivo activity of antimicrobials against *Leptospira* spp. are limited to a few agents and studies in humans. Most of the available human treatment data were obtained in four well-designed, randomized trials. Two of these studies evaluated intravenous penicillin in treatment of severe or icteric disease (5, 18), the third evaluated oral doxycycline for treatment of acute febrile illness (9), and the fourth assessed the efficacies of ceftriaxone and penicillin (14). These studies reported decreased symptoms with antimicrobial therapy, including resolution of fever and leptospiruria, but did not document an increased survival rate.

In vitro antimicrobial susceptibility testing has been used on a small scale to examine the activities of numerous drugs against *Leptospira* isolates (3, 10, 12, 15–17). Most previous studies used broth macrodilution methodologies to produce susceptibility results. Only one limited study has documented the use of a microdilution technique to perform susceptibility testing of *Leptospira* spp. (10). That study evaluated the susceptibility of two strains to four antimicrobial compounds.

There is currently no accepted standard methodology for assessing the in vitro activity of antimicrobial agents against *Leptospira* species. A simple and effective method of testing various antimicrobial agents against multiple *Leptospira* spp. would point to potential alternative therapies for leptospirosis that could be carried forward into animal or human trials. We describe the development of a broth microdilution technique using alamarBlue (Trek Diagnostics, Cleveland, Ohio), a growth detector that has been shown to be effective in the microdilution susceptibility testing of other organisms, including yeast and *Mycobacterium* spp. We determined the susceptibilities of 13 *Leptospira* serovars (seven species) to six antimicrobials by this methodology and directly compared the MICs produced to MICs and minimal bactericidal concentrations (MBCs) produced by a macrodilution method.

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## MATERIALS AND METHODS

***Leptospira* isolates.** Stock strains of *Leptospira* spp. were obtained from the Veterinary Command Food and Drug Analysis Laboratory, Fort Sam Houston, Tex. (strains originated at the USDA National Veterinary Services Laboratories, Ames, Iowa). We included the following strains in the present study: *L. biflexa* serovar Patoc (serogroup Semaranga, strain Patoc I), *L. borgpetersenii* serovar Ballum (serogroup Ballum, strain S 102), *L. borgpetersenii* serovar Sejroe (serogroup Sejroe, strain M 84), *L. interrogans* serovar Australis (serogroup Australis, strain Ballico), *L. interrogans* serovar Autumnalis (serogroup Autumnalis, strain Akiyami A), *L. interrogans* serovar Copenhageni (serogroup Icterohaemorrhagiae, strain M 20), *L. interrogans* serovar Grippotyphosa (serogroup Grippotyphosa, strain Andaman), *L. interrogans* serovar Icterohaemorrhagiae (serogroup

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TABLE 1. MICs produced in 11 runs testing antimicrobials against *L. interrogans* serovar Icterohaemorrhagiae by a microdilution technique

| Antimicrobial | No. of isolates at MIC (μg/ml[a]) of: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≥25 | 12.5 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 | 0.2 | 0.1 | 0.05 | 0.02 | ≤0.01 |
| Cefotaxime | | | | | | | | | | | 7 | 4 |
| Doxycycline | | | | | | 2 | 3 | 2 | 4 | | | |
| Penicillin G | | 4 | 5 | | | | | 2 | | | | |
| Chloramphenicol | | | | 1 | 9 | | | 1 | | | | |
| Erythromycin | | | | | | | | | | | | 11 |
| Ciprofloxacin | | | | | | | 2 | 9 | | | | |

[a] For penicillin G, units per milliliter.

TABLE 3. MBCs produced in 11 runs testing antimicrobials against *L. interrogans* serovar Icterohaemorrhagiae by a macrodilution technique

| Antimicrobial | No. of isolates at MBC (μg/ml[a]) of: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≥25 | 12.5 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 | 0.2 | 0.1 | 0.05 | 0.02 | ≤0.01 |
| Cefotaxime | | | | | | 2 | 2 | 1 | 5 | 1 | | |
| Doxycycline | | 1 | 4 | 6 | | | | | | | | |
| Penicillin G | 11 | | | | | | | | | | | |
| Chloramphenicol | 6 | 5 | | | | | | | | | | |
| Erythromycin | | | | | | 1 | 1 | | 1 | 7 | | 1 |
| Ciprofloxacin | | | 4 | 1 | 4 | 2 | | | | | | |

[a] For penicillin G, units per milliliter.

Icterohaemorrhagiae, strain RGA), *L. interrogans* serovar Pomona (serogroup Pomona, strain Pomona), *L. kirschneri* serovar Butembo (serogroup Autumnalis, strain Butembo), *L. noguchii* serovar Fortbragg (serogroup Autumnalis, strain Fort Bragg), *L. santarosai* serovar Alexi (serogroup Pyrogenes, strain HS 616), and *L. weilii* serovar Celledoni (serogroup Celledoni, strain Celledoni). Note that although our isolates originated at the U.S. Department of Agriculture, six of these strains are available from the American Type Culture Collection (ATCC; Manassas, Va.). These include *L. biflexa* serovar Patoc (serogroup Semaranga, strain Patoc I), available as ATCC 23582; *L. interrogans* serovar Autumnalis (serogroup Autumnalis, strain Akiyami A), available as ATCC 23476; *L. interrogans* serovar Icterohaemorrhagiae (serogroup Icterohaemorrhagiae, strain RGA), available as ATCC 43642; *L. interrogans* serovar Pomona (serogroup Pomona, strain Pomona), available as ATCC 23478; *L. kirschneri* serovar Butembo (serogroup Autumnalis, strain Butembo), available as ATCC 23579; and *L. weilii* serovar Celledoni (serogroup Celledoni, strain Celledoni), available as ATCC 43285. All organisms were maintained by continuous culture in Ellinghausen McCullough Johnson Harris (EMJH) medium (Becton Dickinson, Sparks, Md.).

**Antimicrobial agents.** Stock antimicrobial solutions of 1 mg of each active drug/ml (1,000 U of penicillin G/ml) were prepared with reagent-grade powders with solvents and diluents as suggested in National Committee for Clinical Laboratory Standards document M7-A4 (11). Cefotaxime, chloramphenicol, doxycycline, erythromycin, and penicillin G were purchased from Sigma-Aldrich (St. Louis, Mo.), and ciprofloxacin was obtained from its manufacturer (Bayer Corporation, West Haven, Conn.). All stock antimicrobial solutions were stored in one-time-use aliquots at −70°C.

**Quality control and internal validity.** *L. interrogans* serovar Icterohaemorrhagiae was evaluated by the microdilution and macrodilution methods 11 times over numerous days with several different EMJH medium lots to determine the reproducibility of our methods. Each replicate run used an individually prepared inoculum suspension. Two of these 11 runs were performed in conjunction with the susceptibility testing described below.

**Susceptibility testing.** Testing of each combination of strain and drug was performed in parallel runs by each method, microdilution and macrodilution, to compare the variability of results within and between the methods. Two parallel runs were performed at different times to determine the reproducibility of results. Cumulative efficacy is described as the $MIC_{90}$ and $MBC_{90}$, the concentrations at which 90% of the leptospiral isolates are inhibited and killed, respectively.

TABLE 2. MICs produced in 11 runs testing antimicrobials against *L. interrogans* serovar Icterohaemorrhagiae by a macrodilution technique

| Antimicrobial | No. of isolates at MIC (μg/ml[a]) of: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≥25 | 12.5 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 | 0.2 | 0.1 | 0.05 | 0.02 | ≤0.01 |
| Cefotaxime | | | | | | | | | | | | 11 |
| Doxycycline | | | | 1 | | 2 | 2 | 6 | | | | |
| Penicillin G | | 5 | 5 | | 1 | | | | | | | |
| Chloramphenicol | | | 1 | | 4 | 6 | | | | | | |
| Erythromycin | | | | | | | | | | | | 11 |
| Ciprofloxacin | | | | | | | | 2 | 9 | | | |

[a] For penicillin G, units per milliliter.

**Microdilution.** Broth microdilution testing was performed with 96-well, round-bottom microtiter plates. Each plate included positive controls (bacteria without an antimicrobial), negative controls (medium only), and serial twofold dilutions of each of the six antimicrobials, all in EMJH medium. Antimicrobial-containing wells included final concentrations of each drug, ranging from 25.0 to 0.01 μg/ml (units per milliliter for penicillin G). *Leptospira* inoculum was produced from cultures grown for 7 days at 30°C with organism counts determined by use of a Petroff-Hausser counting chamber under dark-field microscopy. Following the addition of a 100-μl inoculum containing $2 \times 10^6$ leptospiral organisms/ml to the antimicrobial-containing and positive control wells, the plates were incubated at 30°C. The concentration was determined by varying the inoculum to achieve the most consistent growth with numerous serovars (data not shown). The final volume of each well was 200 μl. After 3 days of incubation, 20 μl of 10-times-concentrated alamarBlue was added to all wells. AlamarBlue is a cell growth indicator dye that turns from dark blue to bright pink when growing organisms are present. On the fifth day of incubation, the color of each well was documented, and the MIC was recorded as the lowest concentration used that did not result in the blue-to-pink color change. This 3- plus 2-day incubation period scheme was selected after multiple preliminary studies were conducted to determine optimum timing (data not shown). Three days was determined in early studies to be the shortest incubation time in which we could reliably predict that adequate growth would be present in the positive controls. Following this 3-day incubation period, 2 days of further incubation after the addition of alamarBlue produced clearer end points than those achieved after 1 day of further incubation after the addition of this indicator. End points were clear 1 day after the addition of alamarBlue if plates were incubated for 7 days prior to this addition (7- plus 1-day scheme). The latter incubation scheme was not selected, based on the overall time to results, i.e., 8 versus 5 days.

**Macrodilution.** Broth macrodilution MICs and MBCs were obtained with a previously described technique (8). Antimicrobial-containing tubes were prepared to contain serial twofold dilutions of antimicrobials in EMJH medium in final concentrations of 100 to 0.01 μg/ml (100 to 0.01 U/ml for penicillin G). *Leptospira* spp. were added to each tube to a final concentration of $10^6$ organisms/ml (final volume, 2 ml), and then the tubes were incubated at 30°C for 7 days. The drug concentration contained in the lowest-concentration tube without visual growth was recorded as the MIC. MBC testing was performed by transferring 10 μl of fluid from each tube without visible growth into 2 ml of fresh EMJH medium. The lowest antimicrobial concentration that yielded no growth by visual inspection after 3 weeks of incubation at 30°C was documented as the MBC.

## RESULTS

Quality control (internal-validity) assessment of *L. interrogans* serovar Icterohaemorrhagiae showed consistent results (Tables 1, 2, and 3). MICs determined by microdilution fell within 2 dilutions of each other for 61 (92%) of 66 samples. Sixty-three (93%) of 66 MICs determined by the macrodilution technique were within 2 dilutions of each other. Outliers for each technique were produced by the drugs penicillin G, doxycycline, and chloramphenicol. Macrodilution MBCs had greater variability, with 58 (88%) of the 66 runs producing MBCs that fell within 2 dilutions of each other, although the

TABLE 4. Susceptibility of 12 serovars of *Leptospira* spp. to antimicrobials by microdilution and macrodilution[a]

| Species/serovar | Penicillin G | | | Doxycycline | | | Chloramphenicol | | |
|---|---|---|---|---|---|---|---|---|---|
| | MIC | | Macro MBC | MIC | | Macro MBC | MIC | | Macro MBC |
| | Micro | Macro | | Micro | Macro | | Micro | Macro | |
| *L. biflexa*/Patoc | 0.39/1.56 | 3.13/12.5 | 25/100 | 0.78/0.78 | 1.56/0.78 | 25/>100 | 3.13/3.13 | 6.25/1.56 | 50/100 |
| *L. borgpetersenii*/Ballum | 0.10/0.10 | 0.39/0.39 | 25/1.56 | 0.39/0.39 | 0.20/0.10 | 1.56/0.39 | 6.25/1.56 | 6.25/1.56 | 25/3.13 |
| *L. borgpetersenii*/Sejroe | 0.39/0.20 | 0.39/0.10 | 25/>100 | 0.10/0.10 | 0.20/0.10 | 0.39/3.13 | 1.56/3.13 | 1.56/1.56 | 6.25/12.5 |
| *L. interrogans*/Australis | 0.39/0.39 | 3.13/1.56 | 50/100 | 0.39/0.20 | 0.78/0.20 | 3.13/25 | 1.56/1.56 | 1.56/0.39 | 100/50 |
| *L. interrogans*/Autumnalis | 1.56/0.78 | 3.13/3.13 | 50/50 | 0.20/0.10 | 0.20/0.39 | 0.78/3.13 | 3.13/1.56 | 1.56/1.56 | 6.25/25 |
| *L. interrogans*/Copenhageni | ≤0.01/0.02 | 0.10/0.10 | 25/25 | 0.20/0.39 | 0.20/0.78 | 6.25/12.5 | 3.13/0.78 | 6.25/1.56 | 50/100 |
| *L. interrogans*/Grippotyphosa | 0.20/0.20 | 0.39/3.13 | 6.25/100 | 0.20/0.20 | 0.20/0.39 | 0.78/25 | 3.13/3.13 | 1.56/1.56 | 3.13/50 |
| *L. interrogans*/Pomona | 0.20/0.39 | 1.56/1.56 | 50/50 | 0.78/0.39 | 0.78/0.39 | 12.5/50 | 6.25/3.13 | 3.13/1.56 | 100/>100 |
| *L. kirschneri*/Butembo | 3.13/3.13 | 6.25/0.78 | 100/1.56 | 0.20/0.78 | 0.39/0.10 | 0.78/0.10 | 1.56/6.25 | 0.78/1.56 | 3.13/1.56 |
| *L. noguchii*/Fortbragg | 0.05/0.05 | 0.39/0.39 | >100/100 | 0.39/0.20 | 0.10/0.39 | 6.25/25 | 1.56/0.78 | 0.78/0.78 | 50/50 |
| *L. santarosai*/Alexi | 0.78/0.39 | 6.25/0.78 | 100/50 | 1.56/0.20 | 3.13/0.39 | 12.5/1.56 | 6.25/6.25 | 6.25/3.13 | 50/50 |
| *L. weilii*/Celledoni | 0.39/0.20 | 3.13/0.39 | 25/12.5 | 0.78/0.20 | 3.13/0.20 | 25/6.25 | 1.56/0.78 | 6.25/0.78 | 50/12.5 |
| $MIC_{90}$ or $MBC_{90}$ | 1.56 | 3.13 | 100 | 0.78 | 1.56 | 25 | 6.25 | 6.25 | 100 |

[a] Broth microdilution (Micro) and macrodilution (Macro) MICs and macrodilution MBCs (run 1/run 2 for each) are given in micrograms per milliliter (units per milliliter for penicillin G).

majority of results for penicillin G and chloramphenicol fell outside the upper limit of the drug concentration studied.

In our comparison of microdilution MIC, macrodilution MIC, or macrodilution MBC, duplicate results were available for all drug, *Leptospira* sp. isolate, and test combinations. The observed reproducibility between test runs fell within 2 dilutions for 71 (99%) of the 72 duplicate result sets for MICs by the microdilution method (Table 4). The single set not within 2 dilutions fell within 3 dilutions. The MICs by the macrodilution method produced results between test runs that fell within 2 dilutions for 64 (89%) of the 72 duplicate sets. Of the eight remaining duplicate sets, seven fell within 3 dilutions (penicillin G, four sets; doxycycline, two sets; chloramphenicol, one set; ciprofloxacin, one set) and one fell within 4 dilutions (penicillin G). The MIC irregularities noted in the reproducibility of the microdilution and macrodilution methods were not consistent within serovars or between runs. While the MICs determined by the macrodilution method tended to be higher than those obtained by the microdilution method, the $MIC_{90}$s of all six drugs were within 2 dilutions of each other by the two methods. As with the reproducibility, penicillin G, doxycycline, and chloramphenicol were associated with the most variability between the two methods.

The observed reproducibility of MBCs fell within 2 dilutions for 59 (82%) of the 72 duplicate result sets. Of the remaining 13 sets, six were 3 dilutions apart (doxycycline, four sets; chloramphenicol, one set; ciprofloxacin, one set) and seven were 4 or more dilutions from each other (penicillin G, three sets; ciprofloxacin, two sets; doxycycline, one set; chloramphenicol, one set). Penicillin G, doxycycline, chloramphenicol, and ciprofloxacin were noted to have the largest differences between their macrodilution MIC and MBC results.

## DISCUSSION

Leptospirosis is a potentially fatal infection that is often treated empirically due to the lack of rapid and accurate diagnostic testing. This lack necessitates therapeutic decisions that may cover a broad range of infectious diseases as part of the differential diagnosis. Treatment options are limited, as only three drugs have been evaluated in randomized human trials (5, 9, 14, 18). Currently, there is no standard method to assess antimicrobial agents for antileptospiral activity; however, the most frequently employed means is a cumbersome broth macrodilution method. A rapid and convenient in vitro screening tool to assess the activity of numerous antimicrobial agents against various serovars of *Leptospira* could more expeditiously assess potential antimicrobial agents for clinical utility in animals or humans.

We describe a simple, reliable broth microdilution method for evaluating the susceptibility of organisms of the genus *Leptospira* to antimicrobial compounds. This method is comparable to the traditionally employed broth macrodilution technique, but with the advantages of decreased labor intensity, decreased time to results, and better run-to-run reproducibility.

An assessment of the reproducibility of our technique with a single serovar over numerous days with various lots of media appears to have produced consistent results within techniques. In addition, the microdilution technique appears to be relatively similar in reproducibility to the traditional macrodilution technique, with general agreement of MICs. Although outliers existed, these were not associated with the EMJH medium lots used, with the days on which the tests were performed, or with the number of runs performed in a single day.

We evaluated our methodologies with six drugs from different antimicrobial classes, including the traditional antileptospiral drugs penicillin G and doxycycline, chloramphenicol, and representative drugs from the macrolide (erythromycin), broad-spectrum cephalosporin (cefotaxime), and fluoroquinolone (ciprofloxacin) classes. A diverse selection of leptospiral organisms were included in our study to allow us to look for strain-to-strain and species-to-species variation, as serovar-specific data would not be available before commencement of therapy for an acute infection. We included 13 representative serovars from 10 serogroups (seven species). The MICs were

TABLE 4—*Continued*

| Erythromycin | | | Cefotaxime | | | Ciprofloxacin | | |
|---|---|---|---|---|---|---|---|---|
| MIC | | Macro MBC | MIC | | Macro MBC | MIC | | Macro MBC |
| Micro | Macro | | Micro | Macro | | Micro | Macro | |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.05/0.20 | 0.10/0.20 | 0.39/0.10 | 0.39/0.39 | 0.02/≤0.01 | 0.10/0.02 | 0.39/1.56 |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.02/≤0.01 | 0.02/≤0.01 | 0.05/≤0.01 | 0.20/0.20 | 0.78/0.10 | 0.78/0.10 |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.02/≤0.01 | 0.20/0.20 | 0.10/0.10 | 12.5/0.78 |
| ≤0.01/≤0.01 | ≤0.01/0.02 | 0.78/0.20 | ≤0.01/≤0.01 | 0.02/≤0.01 | 0.05/≤0.01 | 0.20/0.10 | 0.20/0.05 | 25/0.39 |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.02/≤0.01 | ≤0.01/0.02 | 0.05/0.05 | 0.20/0.10 | 0.05/0.05 | 0.10/0.05 |
| ≤0.01/≤0.01 | 0.05/≤0.01 | 0.20/0.78 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.02/0.02 | 0.20/0.20 | 0.20/0.10 | 3.13/1.56 |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.02/0.05 | 0.20/0.10 | 0.05/0.05 | 0.10/0.10 |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.20/0.20 | 0.02/0.02 | ≤0.01/0.02 | 0.05/0.10 | 0.39/0.10 | 0.10/0.05 | 0.78/0.20 |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/0.02 | 0.10/0.20 | 0.05/0.05 | 0.10/0.10 | 0.20/0.20 | 0.10/0.20 | 0.20/0.20 |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.20/0.05 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.10/0.02 | 0.10/0.10 | 0.05/0.10 | 12.5/12.5 |
| ≤0.01/≤0.01 | 0.02/≤0.01 | 0.20/0.05 | 0.10/0.10 | 0.05/0.10 | 0.10/0.10 | 0.20/0.39 | 0.20/0.20 | 25/25 |
| ≤0.01/≤0.01 | ≤0.01/≤0.01 | ≤0.01/≤0.01 | 0.05/0.02 | 0.02/≤0.01 | 0.05/0.02 | 0.10/0.10 | 0.20/0.05 | 0.20/0.10 |
| ≤0.01 | 0.02 | 0.20 | 0.10 | 0.10 | 0.10 | 0.20 | 0.20 | 25 |

more reproducible with the microdilution technique (99%) than with the macrodilution technique (89%). The MICs noted in our microdilution technique were generally lower than those seen with the macrodilution technique, although results with the two techniques consistently followed similar trends for matching serovar and drug combinations. Erythromycin and the broad-spectrum cephalosporin cefotaxime were reproducibly shown to have the greatest activity against *Leptospira* sp. serovars with both the microdilution and macrodilution techniques. The MICs of these two drugs and of ciprofloxacin were consistently lower than those observed for the current drugs of choice for leptospirosis, penicillin G and doxycycline. Chloramphenicol typically required the highest concentrations to produce inhibition or a bactericidal effect.

Although this is the first use of the indicator alamarBlue in the study of *Leptospira* species, this indicator has been used with other bacteria, with cell lines, and with yeast species. Specifically, it has been employed in the susceptibility testing of other slow-growing bacterial organisms, including *Mycobacterium tuberculosis* and *Mycobacterium avium* (4, 7, 13). Given the similar results between microdilution and macrodilution detection methods, microdilution appears reliable.

Intravenous penicillin G, ceftriaxone, and oral doxycycline are the drugs currently suggested for use in the therapy for leptospirosis in humans. This selection is based on experience and on the results of the previously noted clinical trials (5, 9, 14, 18). Many small in vitro and in vivo (animal) studies have shown that a variety of antimicrobials have potential value in the therapy for this infection (1–3, 6, 10, 12, 15–17, 19). The major limitations of these studies are the small numbers of antimicrobials and isolates of *Leptospira* spp. examined at one time and by one method. Our broth microdilution antimicrobial susceptibility testing will allow for the efficient screening of a larger number of antimicrobials against a greater range of *Leptospira* sp. serovars and will aid in the selection of promising agents for further assessment in therapeutic models, including animal and human treatment or prophylaxis trials.

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