In Vitro Susceptibility of Isolates of Francisella tularensis Types A and B from North America

Sandra K. Urich and Jeannine M. Petersen*

Bacterial Diseases Branch, Division of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado

Received 7 December 2007/Returned for modification 9 March 2008/Accepted 5 April 2008

Due to concern that Francisella tularensis, the causative agent of tularemia, may be used as a bioterrorist weapon, the Clinical and Laboratory Standards Institute recently provided a susceptibility testing method with breakpoints. Here, 169 isolates (92 type A and 77 type B) from North America were tested against seven antimicrobial agents (streptomycin, gentamicin, tetracycline, doxycycline, ciprofloxacin, levofloxacin, and chloramphenicol) used for the treatment of tularemia. The MICs for all of the isolates fell within the susceptible range. In addition, all isolates had MICs for erythromycin of 0.5 to 4 μg/ml, in contrast to an MIC of >256 μg/ml for the common laboratory strain LVS (live vaccine strain).

Francisella tularensis is the causative agent of tularemia, a zoonosis of the northern hemisphere. In recent years, interest in this bacterium has been heightened because of concern that it may be used as a bioterrorist weapon (4, 9). Humans contract tularemia from rabbits and rodents, biting insects and ticks, and occasionally by inhalation of infectious aerosols. Cases in the United States are sporadic or occur in small clusters (4). In North America, two subspecies, F. tularensis subsp. tularensis (type A) and holarctica (type B), cause disease. Type A has been further subdivided into two subpopulations, A1 and A2, which differ with respect to geographic location and clinical illness in infected humans (11).

F. tularensis has been reported to be susceptible to a variety of antimicrobial agents, including aminoglycosides, chloramphenicol, quinolones, and tetracyclines (12). Streptomycin was established early as the drug of choice for treating tularemia. Treatment with another aminoglycoside, gentamicin, has been more common in more recent years. Bacteriostatic agents such as chloramphenicol and tetracyclines have also been used but are associated with a higher risk of relapse. Quinolones, which have intracellular activity, have been introduced most recently as possible options for treatment.

The purpose of this study was to evaluate a large panel of geographically and temporally diverse F. tularensis isolates from North America against traditional and newer antimicrobial agents. Although several reports assessing antimicrobial susceptibility have been conducted (1, 5, 6, 7, 8, 10, 13), much of these data were not obtained by a Clinical and Laboratory Standards Institute (CLSI)-approved susceptibility method, raising the possibility that resistance went undetected. Recently, CLSI provided a method and breakpoints for antimicrobial susceptibility testing of F. tularensis (2). In this study, 169 F. tularensis strains (92 type A and 77 type B) from North America were tested by the CLSI-recommended broth microdilution procedure for F. tularensis (2, 3).

F. tularensis strains were submitted to the Centers for Disease Control and Prevention from 40 U.S. states and Canada between 1974 and 2005 and maintained at −75°C in brain heart infusion broth with 10% glycerol. The sources of these isolates were humans (n = 143) and animals (rabbits, rodents, and primates; n = 26). Isolates were confirmed as F. tularensis by characteristic growth on cysteine heart agar with 9% cholesteralized sheep blood (CHAB) and direct fluorescent-antibody staining. The subspecies (types A and B) were differentiated by glycerol fermentation with a GN2 microplate and the MicroLog System (Biolog, Inc., Hayward, CA). Type A strains were divided into two subpopulations, A1 and A2, based on PmeI pulsed-field gel electrophoresis subtyping (11). All work with F. tularensis cultures was performed in a biosafety level 3 laboratory with biosafety level 3 safety precautions.

Seven antimicrobial agents with doubling dilutions in their therapeutic ranges were tested: 0.03 to 64 μg/ml for gentamicin, 0.25 to 512 μg/ml for streptomycin, 0.06 to 128 μg/ml for tetracycline, 0.03 to 64 μg/ml for doxycycline, 0.001 to 2 μg/ml for ciprofloxacin, 0.004 to 8 μg/ml for levofloxacin, and 0.12 to 256 μg/ml for chloramphenicol. Erythromycin (0.5 to 256 μg/ml) was added to assess whether any North American strains are resistant to this drug since the MIC of erythromycin is in the resistant range for type B strains in northern Europe and Russia (6, 8, 12, 13). There are no CLSI guidelines for erythromycin with gram-negative organisms, so breakpoints for resistance are unavailable. However, erythromycin sensitivity could aid in identifying F. tularensis subsp. holarctica (type B) isolates from outside North America. Therefore, F. tularensis subsp. holarctica LVS was included as a control in this study since it originates from Russia and is known to be erythromycin resistant.

Broth microdilution plates were prepared with the CLSI-recommended media for F. tularensis (cation-adjusted Mueller Hinton broth supplemented with 2% defined growth supplement [IsoVitaleX], pH 7.3 ± 1) by Trek Diagnostic Systems, Cleveland, OH. Growth and purity control wells were included on all plates. Plates arrived frozen and were stored at −75°C.
until use. Prior to broth microdilution susceptibility testing, *F.
tularensis* isolates were subcultured from frozen stocks onto 
CHAB, followed by two additional subcultures on chocolate 
agar II plates (BD Diagnostic Systems, Sparks, MD) for 48 h at 
35°C. Quality control strains *Escherichia coli* ATCC 25922, 
*Staphylococcus aureus* ATCC 29513, and *Pseudomonas aerugi-
nosa* ATCC 27853, stored at −75°C, were subcultured three 
times to sheep blood agar for 18 to 24 h at 35°C. Inocula were 
prepared by suspending colonies in Mueller-Hinton broth (BD 
Diagnostic Systems) to a 0.5 McFarland standard with a tur-
bidity meter. This suspension was diluted 20-fold, and 10 μl 
was inoculated with broth microdilution inoculators (PML Mi-
crobiologicals, Wilsonville, OR) into freshly thawed broth mi-
crodilution plates at room temperature. Plates were covered 
with adhesive covers, placed into plastic bags, and incubated in 
ambient air at 35°C. The final inoculum concentrations deter-
mined by colony counts from the growth control well were 
~5 × 10⁵ CFU/ml and ~2 × 10⁶ CFU/ml for the quality 
control and *F. tularensis* strains, respectively. The MICs were 
read at 24 and 48 h for quality control strains and at 48 h for 
*F. tularensis* isolates (2). Antimicrobial testing with the quality 
control strains was performed with every batch of *F. tularensis* 
isolates to verify that the results fell within the acceptable 
range (2).

The MIC distributions for the 169 isolates are listed in Table 
1. For the seven antimicrobial agents tested, the MICs fell 
within the susceptible range for *F. tularensis* defined in the 
CLSI standards (2). The MICs that inhibited the growth of 50 
and 90% of the isolates (MIC₅₀ and MIC₉₀, respectively) are 
shown in Table 2. The MIC₅₀s and MIC₉₀s for the two *F.
tularensis* subspecies, type A and type B, were in agreement, 
with variation within 1 doubling dilution, for all seven antibi-
otics. The MIC₉₀s for the A₁ (*n* = 65) and A₂ (*n* = 23) strains 
were also in agreement, with variation within 1 doubling dilu-
tion, for all seven antibiotics. Overall, the most active antimi-
crobial agents in vitro were the fluoroquinolones. 

Regarding erythromycin, all 169 North American strains (*F.
tularensis* types A and B) fell within the MIC range of 0.5 to 4 
μg/ml. The MIC₅₀ and MIC₉₀ for type A and type B strains 
differed by 2 doubling dilutions, with lower MICs for type A 
strains. In contrast to the North American strains, for the 
Russian *F. tularensis* type B strain (LVS), the MIC of erythro-
mycin was >256 μg/ml (not shown). Thus, erythromycin sen-
sitivity differs between isolates from North America and LVS, 
an attenuated strain common in many academic and public 
health laboratories throughout North America. 

Although previous studies have examined the susceptibilities 
of *F. tularensis* isolates to various antimicrobial agents, no

### Table 1. Antimicrobial MIC distributions for 169 *F. tularensis* isolates

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (μg/ml)*</th>
<th>Type B strains</th>
<th>Type A strains</th>
<th>Type B strains</th>
<th>Type A strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001 0.002 0.004 0.008 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128 256 512</td>
<td>0.03–0.5 0.12 0.25 0.03–0.25 0.06 0.12</td>
<td>0.03–0.5 0.12 0.25 0.03–0.25 0.06 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>NT</td>
<td>0.001 0.002 0.004 0.008 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128 256 512</td>
<td>NT</td>
<td>0.001 0.002 0.004 0.008 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128 256 512</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1 1 1 3 69 87 9</td>
<td>NT</td>
<td>0.001 0.002 0.004 0.008 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128 256 512</td>
<td>NT</td>
<td>0.001 0.002 0.004 0.008 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 32 64 128 256 512</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Vertical bars indicate susceptibility breakpoints.
* NT, not tested for susceptibility to that concentration.

### Table 2. In vitro activities of eight antibiotics against 169 isolates of *F. tularensis*

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Type B strains</th>
<th>Type A strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>0.03–0.5 0.12 0.25</td>
<td>0.03–0.25 0.06 0.12</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.25–4 1 2</td>
<td>0.25–4 1 2</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.25–4 1 2</td>
<td>0.25–4 1 2</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>0.25–2 0.5 1</td>
<td>0.12–2 0.5 1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.25–4 1 2</td>
<td>0.25–4 1 2</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.004–0.06 0.03 0.06</td>
<td>0.008–0.06 0.015 0.03</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.015–0.12 0.03 0.06</td>
<td>0.015–0.12 0.03 0.06</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.5–4 2 2</td>
<td>0.5–4 2 2</td>
</tr>
<tr>
<td>Phenicols</td>
<td>0.5–4 2 2</td>
<td>0.5–4 2 2</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.5–4 2 2</td>
<td>0.5–4 2 2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.5–4 2 2</td>
<td>0.5–4 2 2</td>
</tr>
</tbody>
</table>

* Values are in micrograms per milliliter.
standardized method of testing or interpretative criteria had been established. In 2006, CLSI provided a broth microdilution method for \textit{F. tularensis} with breakpoints including interpretative criteria for quality control organisms. With this standardized methodology, no antimicrobial resistance to seven antimicrobial agents (streptomycin, gentamicin, tetracycline, doxycycline, ciprofloxacin, levofloxacin, and chloramphenicol) used for treatment was detected in North American strains. These results are consistent with other antimicrobial studies performed with \textit{F. tularensis} isolates from throughout the northern hemisphere and the fact that treatment failure due to resistance of \textit{F. tularensis} to the antibiotics used for clinical therapy has never been demonstrated (1, 5, 6, 7, 8, 10, 12, 13).

We acknowledge Kristy Kubota, Aimee Janusz, and Kiersten Kugeler for their assistance in performing pulsed-field gel electrophoresis analysis on type A strains in this study and Marty Schriefer for critical review of the manuscript.

We also acknowledge all of the state and local health departments that obtained isolates used in this study.

**REFERENCES**