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Antimicrobial Susceptibility of *Francisella tularensis* Isolates in the United States—2009–2018

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Abstract

Francisella tularensis is the causative agent of tularemia. We tested the susceptibility of 278 *F. tularensis* isolates from the United States received during 2009–2018 to eight antimicrobial drugs (ciprofloxacin, levofloxacin, doxycycline, tetracycline, gentamicin, streptomycin, chloramphenicol, and erythromycin). All isolates were susceptible to all tested drugs.

Keywords

antimicrobial susceptibility; minimum inhibitory concentration; *Francisella tularensis*

Infection with the bacterium *Francisella tularensis* causes the disease tularemia. *F. tularensis* is considered a potential bioweapon due to its high level of infectiousness, and is classified as a Tier 1 select agent, considered to pose the highest risk of misuse. Inhaling as few as 10 organisms can cause disease [1]. Natural infection occurs through environmental exposures such as direct contact with infected rodents or contaminated water or through bites of vectors such as tick and deer flies [2]. Human to human transmission has not been reported. Two subspecies of *F. tularensis,* subsp. *tularensis* (Type A) and subsp. *holarctica* (Type B), cause tularemia. A third subspecies, subsp. *mediasiatica*, has not been associated with human disease. Type A is found only in North America and is associated with more severe infections, while Type B is found throughout the Northern Hemisphere and has also been reported in Australia [2, 3]. Within Type B, there are three biovars, differentiated in part by erythromycin sensitivity [2, 4, 5].

F. tularensis is susceptible to multiple antimicrobial agents used for treatment of tularemia, including tetracyclines, aminoglycosides, and fluoroquinolones [5–8]. The Clinical and Laboratory Standards Institute (CSLI) defines methods and breakpoints for *F. tularensis* susceptibility by broth microdilution to seven antimicrobial agents:

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the fluoroquinolones ciprofloxacin and levofloxacin; the tetracyclines doxycycline and tetracycline; the aminoglycosides gentamicin and streptomycin; and chloramphenicol [9]. Breakpoints have not been defined for susceptibility to erythromycin. Nonetheless, we included it in this study to determine if resistance was detectable in isolates from the United States.

In this study, we report on the minimum inhibitory concentrations (MICs) of 278 *F. tularensis* isolates from human and animal sources received by the CDC from 33 US states during 2009–2018. The 278 isolates consist of 168 *F. tularensis* subsp. *tularensis* (Type A) isolates and 110 *F. tularensis* subsp. *holarctica* (Type B) isolates. 217 isolates were derived from human clinical samples, and 61 were isolated from non-human animals. We grew each isolate on cysteine heart agar with 9% chocolatized sheep blood (CHAB) and confirmed its identity as *F. tularensis* using a direct fluorescent antibody assay. All *F. tularensis* culture work was performed in a biosafety level 3 (BSL-3) laboratory with BSL-3 safety precautions.

Custom broth microdilution MIC panels prepared by Thermo Fisher Scientific (Thermo Fisher Scientific, Oakwood Village, OH) were used to assess the susceptibility of *F. tularensis* isolates and controls. Each plate includes eight different antimicrobial agents with doubling dilutions covering therapeutic ranges in cation-adjusted Mueller Hinton broth with 2% defined growth supplement (IsoVitaleX). The ranges are 0.001–2 μg/mL for ciprofloxacin, 0.004–8 μg/mL for levofloxacin, 0.03–64 μg/mL for doxycycline, 0.06–128 μg/mL for tetracycline, 0.03–64 μg/mL for gentamicin, 0.25–512 μg/mL for streptomycin, 0.12–256 μg/mL for chloramphenicol, and 0.5–256 μg/mL for erythromycin (Figure 1). Each plate also contains a positive (no antimicrobial agent) and negative (water) growth control well.

On each day of testing, we tested three quality control (QC) organisms in parallel: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213. Prior to *F. tularensis* inoculation on the MIC plate, we subcultured each *F. tularensis* isolate twice on CHAB and incubated the plates at 35°C for 24–48 hours. After growth of all organisms, we suspended 3–5 isolated colonies into sterile Mueller-Hinton broth (BD Diagnostic Systems) to a turbidity equivalent to a 0.5 McFarland standard. Then, we inoculated each well of an MIC plate with 10μL of a 1:20 dilution of the suspension using broth microdilution inoculators (PML Microbiologicals, Wilsonville, OR). We recorded MIC values after 24 and 48 hours for each control, and after 48 hours for each *F. tularensis* strain. Data analysis was performed in R 4.1.2 [10].

The MIC values for all 278 *F. tularensis* isolates fell within the susceptible ranges defined by the CSLI guidelines for the antimicrobial agents for which susceptibility breakpoints have been defined (Figure 1; Table 1). The MICs that inhibited the growth of 50 and 90% of the isolates (MIC_{50} and MIC_{90}, respectively) are shown in Figure 1. The susceptibility of Type A and Type B strains was generally similar, with no more than one dilution difference in MIC_{50} or MIC_{90} values between subspecies for any drug.

Although a susceptibility breakpoint has not been defined for erythromycin, all isolates had MICs ≤8 μg/mL, consistent with other data on isolates from the United States and Western Europe [5, 6]. In contrast, the live vaccine strain LVS, which is a Type B strain that originates from Russia, had an erythromycin MIC of >256. Although erythromycin is not recommended for tularemia treatment due to resistance in Eurasian strains, these results indicate that it may be effective for treatment of naturally occurring, locally acquired cases in the United States, where no resistance has been seen.

Other *Francisella* species cause rare, opportunistic human disease [11, 12]. These include *F. novicida* (sometimes referred to as a subspecies of *F. tularensis* based on sequence similarity) and *F. philomiragia*. We performed antimicrobial susceptibility testing on five isolates of *F. novicida* and one isolate of *F. philomiragia*, following the same procedures established for *F. tularensis*. The MICs for all these isolates fell within the susceptibility ranges defined for *F. tularensis*. MICs ranged from 0.015 to 0.06 μg/mL for ciprofloxacin; 0.03–0.06 μg/mL for levofloxacin; 2–4 μg/mL for doxycycline and tetracycline; 0.06–0.12 μg/mL for gentamicin; 0.5–4 μg/mL for streptomycin; 4–8 μg/mL for chloramphenicol, and 1 to 16 μg/mL for erythromycin. Although limited data are available regarding antimicrobial susceptibility in *Francisella* species other than *F. tularensis*, there is no evidence of resistance to drugs recommended for treatment of tularemia in these species.

This study is limited by a lack of *F. tularensis* isolates recovered from 2019 to present. However, the data are consistent with a previous U.S. study that examined strains received during 1974–2005 [6]. Furthermore, as tularemia is generally not transmitted between humans, there is no clear biological mechanism that might drive a change in susceptibility over time. An additional limitation is that this study contains only isolates from the United States. However, the data are consistent with studies from other countries [5, 7, 13–15]. Other than erythromycin, lineage-specific differences in susceptibility of *F. tularensis* have not been reported. Furthermore, this study examined a large number of Type A strains, which occur only in North America and are more virulent than Type B and therefore of higher concern for a biothreat event.

Monitoring antimicrobial susceptibility in both Type A and Type B strains is essential to ensure that recommendations for treatment of tularemia are appropriate. Within this study, no antimicrobial resistance was detected in *F. tularensis* Type A and Type B isolates received between 2009–2018 by the CDC to drugs recommended for treatment of tularemia, including all seven antimicrobial agents listed in CLSI guidelines (chloramphenicol, ciprofloxacin, doxycycline, gentamicin, levofloxacin, streptomycin, and tetracycline), although some isolates were within one dilution of the susceptibility breakpoint for doxycycline and tetracycline. These results remain consistent with previous data and with the lack of reports of treatment failure due to antimicrobial resistance.

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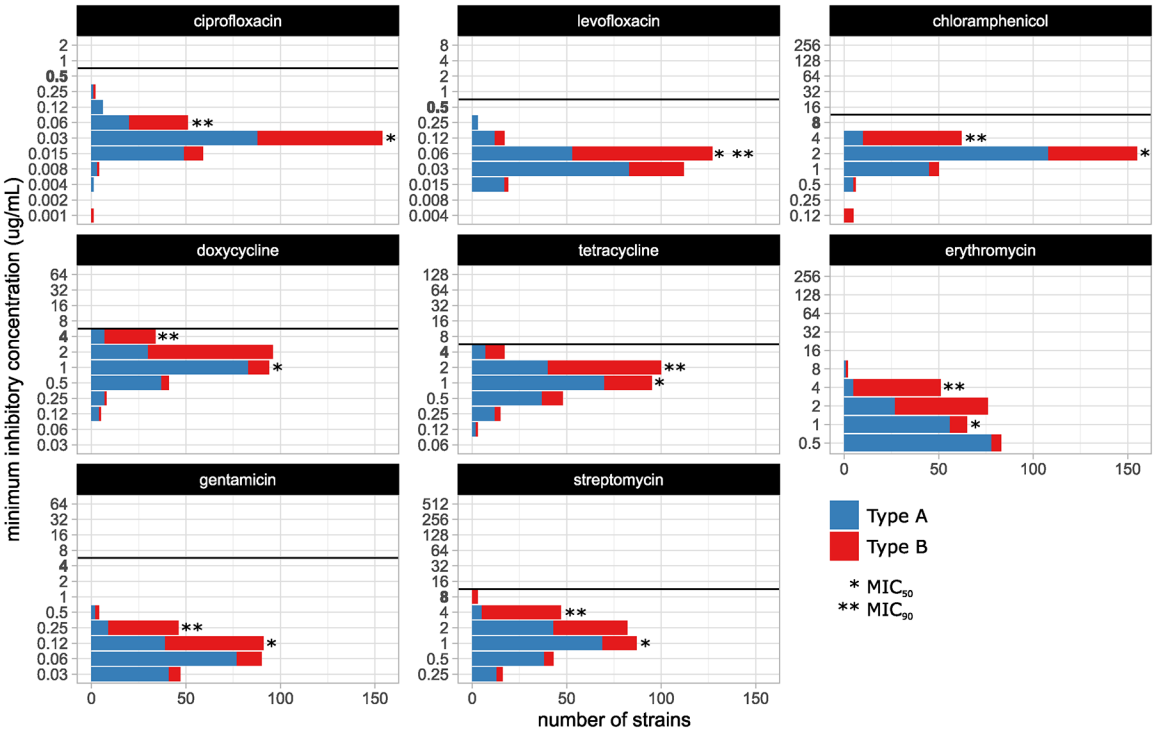

Figure 1.

Distribution of minimum inhibitory concentrations of eight drugs against *F. tularensis* isolates received by CDC during 2009–2018. Susceptibility breakpoints defined by CLSI are marked in bold text on the y-axis, and horizontal lines are placed between the breakpoint MIC and the next doubling dilution [9]. Type A (subspecies *tularensis*) and Type B (subspecies *holarctica*) are represented by blue and red bars, respectively. MIC_{50} and MIC_{90} values are represented by single and double asterisks, respectively.

Table 1.

MIC distributions[a]

Antimicrobial drug	MIC (μg/mL)																			
	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
Ciprofloxacin	1	0	1	4	59	154	51	6	2	0	0	0								
Levofloxacin			0	0	19	112	127	17	3	0	0	0	0	0						
Doxycycline						1	0	5	8	41	93	96	34	0	0	0	0			
Tetracycline							0	3	15	48	95	100	17	0	0	0	0	0		
Gentamicin						47	90	91	46	4	0	0	0	0	0	0	0			
Streptomycin									16	43	87	82	47	3	0	0	0	0	0	0
Chloramphenicol								5	0	6	50	155	62	0	0	0	0	0	0	
Erythromycin										83	65	76	51	2	0	0	0	0	0	

[a]Black filled boxes indicate that testing was not performed.

Bold vertical bars indicate CLSI susceptibility breakpoints.

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