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Short Communication

In Vitro Antibiotic Susceptibility of *Francisella tularensis* Isolates from Japan

Akitoyo Hotta[1]*, Osamu Fujita[1], Akihiko Uda[1], Neekun Sharma[1,2], Kiyoshi Tanabayashi[1], Yoshie Yamamoto[1], Akio Yamada[3], and Shigeru Morikawa[1,2]

[1]*Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo 162-8640;* [2]*The United Graduate School of Veterinary Science Gifu University, Gifu 501-1193; and* [3]*Laboratory of Veterinary Public Health, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan*

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SUMMARY: The antibiotic susceptibilities of 36 isolates of Japanese *Francisella tularensis*, an etiological agent of the zoonotic disease tularemia, were analyzed using the E test. All the isolates were susceptible to ciprofloxacin, doxycycline, erythromycin, and gentamicin but resistant to benzylpenicillin and cephalothin. The susceptibility to seven other β-lactams (aztreonam, cefotaxime, cefoxitin, ceftriaxone, cefuroxime, imipenem, and meropenem) varied among the isolates. These findings suggest that the guidelines for the antibiotic treatment of tularemia issued by the World Health Organization are appropriate for Japanese tularemia patients.

Francisella tularensis is an intracellular gram-negative, fastidious coccobacillus that causes the zoonotic disease tularemia in humans and animals. It is widely distributed throughout the northern hemisphere, including Japan (1). Its low infectious dose, ease of dissemination, and history as a bioweapon have led to its inclusion in the Centers for Disease Control and Prevention's (CDC's) Category A select agents (2). Humans can be infected with *F. tularensis* through direct contact with infected animals, arthropod bites, ingestion of contaminated water or food, or inhalation of infectious aerosols. Predominant symptoms are high fever, lymph node enlargement, and ulceration at the site of bacterial entry. Aminoglycosides, chloramphenicol, tetracyclines, and quinolones are recommended for antibiotic treatment (1,3).

Approximately 1,300 cases of human tularemia were reported in Japan between 1924 and 1985, although the incidence of tularemia and isolation of the pathogens have been extremely rare since 1990 (4). In 2008, five cases of tularemia were reported in the Infectious Diseases Weekly Report Japan database (http://idsc.nih.go.jp/idwr/ydata/report-Ea.html) and the pathogen was isolated from a Japanese hare (5). This was the first isolation of *F. tularensis* in Japan in 19 years to the best of our knowledge. Because *F. tularensis* isolates from Japan are known to differ from those from North America and Europe in terms of their biochemical (1) and genetic properties (6), analysis of other characteristics of Japanese isolates is important.

Information on the antibiotic susceptibility pattern of *F. tularensis* is necessary to ensure appropriate prescription of antibiotics for postexposure prophylaxis and therapy for tularemia patients. Recently, the antibiotic susceptibilities of *F. tularensis* isolates from North America (7), Austria (8), France (9), and Turkey (10) were analyzed using the E test. Those of *F. tularensis* isolates from Japan were analyzed using a conventional agar dilution method (11). In the present study, the antibiotic susceptibilities of Japanese *F. tularensis* isolates were analyzed using the E test and ready-made agar plates. These data would be helpful for comparing the antibiotic susceptibility of each isolate stored among different laboratories.

Thirty-four isolates of Japanese *F. tularensis* that were collected between 1926 and 1989 (Aichi, Azumaya, Chiba, Ebina, GIEM Miura, Hashimoto, Himizu, Hitosu, Ito, Jap, Kato, Kawamata, Kikuchi, Kokuchi, Metomo, Mitsuo, Murayama, Naomatsu, Nikaido, Oniwa, Ootake, Sami, Sashige, Shinomiya, Suzushichi, Takahashi, Tateyama, TH, TI, Tsuchiya, Yato11, Yato96, Yato107, and Yama) were kindly provided by Dr. H. Fujita (Ohara Research Laboratories, Fukushima, Japan). These isolates were originated from humans (27 isolates), hares (3 isolates), ticks (3 isolates), and Japanese shrew mole (1 isolate) (6). Two recent isolates, KU-1, a generous gift from Prof. H. Sato (Kitasato University, Aomori, Japan) (5), and NVF1, were isolated from wild hare carcasses in 2008 and 2009, respectively (12). All the *F. tularensis* isolates were grown on Eugon agar plates containing 8% (w/w) chocolatized sheep blood (13) and passaged twice in Mueller–Hinton broth (BD, Sparks, Md., USA) supplemented with 0.1% (w/v) glucose, 2% (v/v) IsoVitalX (BD), and 0.025% (w/v) ferric pyrophosphate (14). *F. tularensis* requires cysteine for growth; therefore, the subcultured bacteria were inoculated on chocolate II agar plates (BD, Fukushima, Japan). The E test strips (AB Biomerieux, Solna, Sweden), including the 13 antibiotics listed in Table 1, were placed on these plates. After incubation at 37°C for 2 days, the minimum inhibitory concentration (MIC) was determined as the

*Corresponding author: Mailing address: Department of Veterinary Science, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku, Tokyo 162-8640, Japan. Tel: +81-3-5285-1111, E-mail: ahotta@nih.go.jp

Table 1. MICs of quality control strains and reference strain determined by use of E test

Antibiotic	MIC (mg/L)					
	E. coli ATCC 25922		*S. aureus* ATCC 29513		*F. tularensis* LVS	
	This study	Ref.[1)]	This study	Ref.	This study	Ref.
Benzylpenicillin	NT		1–1.5	0.25–1	>256	
Cephalothin	8–12	4–16	0.19	0.125–0.5	>256	
Cefuroxime	2–3	2–8	0.75–1	0.5–2	2	
Cefotaxime	0.064–0.094	0.032–0.125	0.75–1	1–4	0.19	
Ceftriaxone	0.032–0.047	0.032–0.125	NT		0.032	0.032
Cefoxitin	2	1–4	NT		0.19	
Imipenem	0.094–0.125	0.064–0.25	NT		0.5	0.5
Meropenem	0.016–0.023	0.008–0.064	NT		0.094	0.38
Aztreonam	0.064	0.064–0.25	NT		0.75	
Erythromycin	NT		0.25–0.38	0.125–0.5	>256	>256
Doxycycline	2–3	0.5–2	1–1.5	0.064–0.25	0.19	0.125–0.25
Gentamicin	2–3	0.25–1	NT		0.064	0.032–0.38
Ciprofloxacin	NT		0.25–0.38	0.125–0.5	0.003	0.006–0.008

[1)]: References on instruction to users of E test or the results of other equivalent reports (7,10,15).
NT, not tested.

point of intersection between the zone of bacterial growth observed and the E test strip. If bacterial growth occurred along the entire strip, i.e., no inhibition ellipse was observed, MIC was recorded to be more than the highest value on the MIC scale for each E test strip (9). *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29513) were used as quality control strains. In addition, *F. tularensis* LVS, which was provided by Dr. H. Fujita, was tested because it is often used as a *F. tularensis* reference strain (7,10,15). A P/Case test-N (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) was used to analyze β-lactamase production by each isolate, according to the manufacturer's instructions. Studies related to *F. tularensis* cultures were performed in a biosafety level 3 laboratory at the National Institute of Infectious Diseases, Tokyo, Japan.

MICs of the quality control strains (*E. coli* and *S. aureus*) and *F. tularensis* LVS were similar to the reference MICs found in the instructions of the E test, as reported by Valade et al. (9), and MICs reported previously (7,10,15) (Table 1). Using this assay system, ciprofloxacin, doxycycline, erythromycin, and gentamicin effectively inhibited the growth of all isolates with MIC ranges of 0.003–0.023, 0.094–1.5, 0.094–1.5, and 0.023–0.5 mg/L, respectively (Table 2). These results showed that the susceptibilities of the isolates from Japan to these four antibiotics were similar to those of the isolates from North America (7) but different from those of the isolates from Turkey (10) and Austria (8), particularly with regard to susceptibility to erythromycin.

The other nine tested β-lactams antibiotics (aztreonam, benzylpenicillin, cefotaxime, cefoxitin, ceftriaxone, cefuroxime, cephalothin, imipenem, and meropenem) did not effectively inhibit the growth of the tested isolates. Three isolates (including the recent isolates KU–1 and NVF1) grew over the entire chocolate II agar plate, whereas others showed inhibition ellipses around the E test strips with some of these antibiotics. The MIC results indicated that these nine β-lactams would be ineffective for the treatment of tularemia patients, while the MIC distribution suggested that some Japanese isolates were susceptible to cephalosporins, cephamycin, carbapenems, and monobactam (Table 2). Our results and those of previous reports (11,14,16) suggest that cephalosporin-susceptible *F. tularensis* isolates are distributed in Japan and North America. Cephalosporins are frequently used to clinically treat patients with suspected bacterial infections (17). The early clinical symptoms of human tularemia are flu-like; therefore, its diagnosis is difficult without laboratory tests. There is a possibility that some patients who are exposed to *F. tularensis* and treated with cephalosporins are abandoned without being suspected of and diagnosed as having tularemia. Careful diagnosis and analyses of clinical isolates will be necessary because in vitro antibiotic susceptibility of *F. tularensis* does not always correlate with clinical response (18).

Susceptibility of the isolates to β-lactams does not seem to be related to their origin, such as the host, source, isolated area, and isolation year (data not shown). To clarify the mechanisms underlying the susceptibilities of the isolates to different β-lactams, bacterial β-lactamase (penicillinase and cephalosporinase) production was analyzed using the P/Case test-N. The results showed that all the tested isolates produced penicillinase but not cephalosporinase (data not shown). Thus, the mechanism underlying the differences in susceptibility to β-lactams was not clarified during this analysis. It is possible that *F. tularensis* possesses an unknown antibiotic resistance mechanism.

Our results suggested that the guidelines for the antibiotic treatment of tularemia issued by the World Health Organization (19) are appropriate for Japanese tularemia patients. These findings provide important information related to recommendations for therapy for Japanese tularemia patients as well as for postexposure prophylaxis because *F. tularensis* is designated CDC's Category A bioterrorism agent (3).

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Table 2. MIC distribution of Japanese *Francisella tularensis* isolates

Antibiotic		MIC range[1]	MIC (mg/L)																																		Not inhibited[2]
			0.002	0.003	0.004	0.006	0.008	0.012	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	6	8	12	16	24	32	48	64	96	128	192	256	
Penicillin	Benzylpenicillin	0.016–256																															1					35
Cephalosporins	Cephalothin	0.016–256																															1	2				33
	Cefuroxime	0.016–256																	2	1	1		2			3	1	5		2	2	1						16
	Cefotaxime	0.016–256										1	2	2	2		1		2		1		1															24
	Ceftriaxone	0.002–32										1	1		3	2	2	3	1	3	2																	18
Cephamycin	Cefoxitin	0.016–256															1	3	1	1	5	1	5	4	4	3	1	2										5
Carbapenems	Imipenem	0.002–32										1		1	1	1	1	6	5	5	2	1																12
	Meropenem	0.002–32												2	1	2	1	1		3	4	2	2															18
Monobactam	Aztreonam	0.016–256																		2	2	2	2	3	3	3	3	2		1								13
Macrolide	Erythromycin	0.016–256												1	4	4	2	7	3	8	3	4																0
Tetracycline	Doxycycline	0.016–256												2	3	5	4	4	9	5	2	2																0
Aminoglycoside	Gentamicin	0.016–256								4	2	8	9	5	5	1			2																			0
Fluoroquinolone	Ciprofloxacin	0.002–32		3	7	9	6	7	3	1																												0

[1]: MIC ranges determiend by use of E test.
[2]: Number of isolates that did not show any inhibition ellipse.

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Conflict of interest None to declare.

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