The Class A β -Lactamase FTU-1 Is Native to *Francisella tularensis*

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The class A β -lactamase FTU-1 produces resistance to penicillins and ceftazidime but not to any other β -lactam antibiotics tested. FTU-1 hydrolyzes penicillin antibiotics with catalytic efficiencies of 10^5 to 10^6 $M^{-1} s^{-1}$ and cephalosporins and carbapenems with catalytic efficiencies of 10^2 to 10^3 $M^{-1} s^{-1}$, but the monobactam aztreonam and the cephamycin cefoxitin are not substrates for the enzyme. FTU-1 shares 21 to 34% amino acid sequence identity with other class A β -lactamases and harbors two cysteine residues conserved in all class A carbapenemases. FTU-1 is the first weak class A carbapenemase that is native to *Francisella tularensis*.

Since the discovery of β -lactams more than a half-century ago, they continue to be the major class of antibiotics used in the treatment of infectious diseases (31). Their intensive use has resulted in the selection of microorganisms that produce β -lactamases, enzymes which hydrolyze β -lactam antibiotics, thus significantly narrowing our therapeutic options (14). Based on their amino acid sequence identity and mechanisms of catalysis, β -lactamases are subdivided into four major classes: A, B, C, and D. β -Lactamases from classes A, C, and D employ an active-site serine while class B enzymes utilize metal, usually Zn^{2+} , for catalysis (4, 14).

Shortly after the introduction of β -lactams into clinical use, class A β -lactamases were identified which were so-called non-extended-spectrum enzymes, capable of hydrolyzing only penicillins and early-generation cephalosporins (35). In response to the subsequent introduction of third-generation cephalosporins and β -lactamase inhibitors, extended-spectrum β -lactamases (ESBLs) and inhibitor-resistant (IRT) enzymes have been selected (5, 22, 35). These enzymes evolved from their non-extended-spectrum progenitors as a result of only a few mutations or, in some cases, with just one, widening their substrate specificity (27). Currently ESBLs are widely distributed among Gram-negative clinical isolates and are capable of inactivating the majority of available β -lactam antibiotics, with the notable exception of carbapenems (25, 35).

While use of carbapenems in the treatment of serious infections caused by ESBLs and IRT enzymes failed to select for carbapenem-resistant variants of these enzymes, novel class A β -lactamases of unknown origin and capable of hydrolyzing carbapenem antibiotics have been selected (35, 42). These enzymes have relatively low amino acid identity with “classical” class A β -lactamases and harbor two characteristic cysteine residues at Ambler positions 69, directly preceding the active site serine, and 238, which follows two residues after the conserved KTG motif (42). These cysteine residues form a disulfide bridge which serves to connect the two domains of the enzyme. Currently, several subfamilies of class A enzymes containing these characteristic cysteine residues have been described. They include the IMI/NMC-A, GES-, KPC-, BEL-, and SME-type enzymes (36, 37, 41), as well as SFC-1 (26) and BIC-1 (21). Though structurally all these enzymes belong to the class A carbapenemase family, some of them, such as GES-1 and BEL-1, do not elevate the MICs of carbapenem antibiotics and are devoid of or exhibit very low carbapenemase activity (36, 38). Here, we report a class A β -lactamase from *Francisella*

tularensis subsp. *holarctica* LVS, which we call FTU-1, that contains the conserved cysteine residues characteristic of the class A carbapenemase family. Unlike all previously reported class A carbapenemases which are found only sporadically in various clinical and environmental microorganisms, FTU-1 is a native enzyme encoded by a chromosomally located gene in all *Francisella tularensis* strains.

MATERIALS AND METHODS

Strains and plasmids. The open reading frame encoding a putative class A β -lactamase from *F. tularensis* subsp. *holarctica* LVS was used to construct a synthetic gene for the FTU-1 enzyme. The nucleotide sequence of the synthetic gene was optimized for expression in *Escherichia coli*, and the predicted leader sequence was replaced by that for the outer membrane protein OmpA (12). MIC determinations were performed using *E. coli* JM83 carrying the FTU-1 gene cloned between the unique NdeI and HindIII sites of pHF016 (16). The enzyme was expressed and purified from *E. coli* BL21(DE3) harboring the FTU-1 gene cloned between the unique NdeI and HindIII sites of pET24a(+) (Invitrogen).

Antimicrobial susceptibility testing. The susceptibility to several β -lactam antimicrobials and their combinations with β -lactamase inhibitors was determined by the broth microdilution method as described by the Clinical and Laboratory Standards Institute guidelines (8). The MICs were determined in triplicate using Mueller-Hinton II broth (Difco) and a bacterial inoculum of 5×10^5 CFU/ml. The plates were incubated at 37°C for a period of 16 to 20 h before the results were interpreted.

Enzyme purification. *E. coli* BL23(DE3) carrying the gene for FTU-1 cloned into pET24a(+) was grown at 37°C with shaking (180 rpm) in LB broth supplemented with 0.5 M sorbitol, 25 mM betaine, and 60 μ g/ml kanamycin. When the bacterial culture reached an optical density of 0.8 at 600 nm, isopropyl- β -D-thiogalactopyranoside was added to a final concentration of 0.4 mM, and the bacteria were further grown for 20 h at 30°C with shaking. The culture was pelleted by centrifugation at $20,000 \times g$ and 4°C for 30 min. The broth was recovered and concentrated by centrifugal filtration using a Centricon Plus 70 concentrator (Millipore) with a molecular-mass-cutoff of 10 kDa. After overnight dialysis into 20 mM MES (pH 6.0), the sample was loaded onto a DEAE anion-exchange column (Bio-Rad) equilibrated with the same buffer. At this pH, FTU-1 does

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not bind to the resin and can be recovered in the flowthrough. Two additional column volumes of 20 mM morpholineethanesulfonic acid ([MES] pH 6.0) were added, and the flowthrough was collected in 10-ml fractions. The fractions containing FTU-1 were identified by their activity against the chromogenic β -lactam nitrocefin and combined. After concentration, the enzyme was dialyzed against 20 mM Tris (pH 8.0) and loaded onto a MacroPrep S cation-exchange column (Bio-Rad), equilibrated in the same buffer. The column was washed with three column volumes of 20 mM Tris (pH 8.0), and FTU-1 was eluted using a linear gradient of NaCl (0 to 500 mM). The fractions containing the enzyme were identified by their activity toward nitrocefin, and their purity was assessed by SDS-PAGE prior to dialysis into 20 mM HEPES (pH 7.5) for storage. The protein concentration was determined using the predicted extinction coefficient ($\Delta\epsilon_{280} = 30,495 \text{ cm}^{-1} \text{ M}^{-1}$) (20).

Determination of steady-state kinetic parameters. All spectrophotometric data were collected on a Cary 50 spectrophotometer (Varian). The reactions were performed at room temperature in 50 mM sodium phosphate (pH 7.0). The following extinction coefficients and wavelengths were used: ampicillin, $\Delta\epsilon_{235}$ of $-670 \text{ cm}^{-1} \text{ M}^{-1}$; benzylpenicillin, $\Delta\epsilon_{232}$ of $-1,096 \text{ cm}^{-1} \text{ M}^{-1}$; oxacillin, $\Delta\epsilon_{260}$ of $440 \text{ cm}^{-1} \text{ M}^{-1}$; cefotaxime, $\Delta\epsilon_{265}$ of $-6,643 \text{ cm}^{-1} \text{ M}^{-1}$; cefoxitin, $\Delta\epsilon_{265}$ of $-7,507 \text{ cm}^{-1} \text{ M}^{-1}$; cef-tazidime, $\Delta\epsilon_{260}$ of $-10,500 \text{ cm}^{-1} \text{ M}^{-1}$; cefuroxime, $\Delta\epsilon_{262}$ of $-7,800 \text{ cm}^{-1} \text{ M}^{-1}$; cephalothin, $\Delta\epsilon_{262}$ of $-8,610 \text{ cm}^{-1} \text{ M}^{-1}$; aztreonam, $\Delta\epsilon_{318}$ of $-640 \text{ cm}^{-1} \text{ M}^{-1}$; imipenem, $\Delta\epsilon_{297}$ of $-10,930 \text{ cm}^{-1} \text{ M}^{-1}$; meropenem, $\Delta\epsilon_{298}$ of $-7,200 \text{ cm}^{-1} \text{ M}^{-1}$; doripenem, $\Delta\epsilon_{299}$ of $-11,460 \text{ cm}^{-1} \text{ M}^{-1}$; and ertapenem, $\Delta\epsilon_{295}$ of $-9,970 \text{ cm}^{-1} \text{ M}^{-1}$. The steady-state velocities were determined from the linear phase of the reaction time courses and plotted as a function of β -lactam concentration to allow determination of both k_{cat} and K_m by nonlinear regression using the Michaelis-Menten equation and Prism, version 5 (GraphPad Software, Inc.). The half-life of a β -lactam was evaluated as the $\ln 2/k_{\text{cat}}$. When K_m was high and saturation could not be reached, the ratio k_{cat}/K_m was determined by fitting the reaction time course with equation 1:

$$A_t = A_{\infty} + A_0 e^{-kt} \quad (1)$$

where A_t is the absorbance at time t , A_{∞} is the final absorbance, A_0 is the initial absorbance, and $k = (k_{\text{cat}}/K_m) [E]$, where $[E]$ is the concentration of enzyme (9).

Determination of inhibitor dissociation constants. The inhibitor dissociation constants, K_i , for the inhibitors clavulanic acid, tazobactam, and sulbactam were determined using nitrocefin ($\Delta\epsilon_{500} = 15,900 \text{ cm}^{-1} \text{ M}^{-1}$) as a reporter substrate. The reactions were made in 50 mM NaP_i (pH 7.0), containing nitrocefin (40 and 60 μM) and several concentrations of the inhibitor (0 to 1 mM), and initiated by the addition of the enzyme (10 nM). The reactions were followed for 2 min at 500 nm, and the time courses were fit with equation 2 (9):

$$A_t = v_{\text{ss}} t + \frac{v_i - v_{\text{ss}}}{k_{\text{obs}}} (1 - e^{-k_{\text{obs}} t}) + A_0 \quad (2)$$

where A_t represents the absorbance at time t , A_0 is the absorbance at time zero, v_i is the initial velocity, v_{ss} is the steady-state velocity, and k_{obs} is the rate constant for the interconversion between v_i and v_{ss} . The value for K_i was determined by the method of Dixon (10).

RESULTS AND DISCUSSION

Analysis of *F. tularensis* genomes. In search of novel class A carbapenemases, we analyzed sequences of proteins encoded by strains of *F. tularensis* whose whole genomes had been sequenced and deposited in the BioProject Database of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/bioproject>). We screened for class A β -lactamases that contained two conserved cysteine residues, Cys69 and Cys238, a characteristic feature of all carbapenemases. Our analysis demonstrates that of the 14 genomes of *F. tularensis* strains currently deposited in the database, including representatives from all four known *F. tular-*

TABLE 1 List of available *F. tularensis* genomes and putative class A carbapenemases

Strain (reference[s])	Genome accession no.	Geographical origin	Isolate type	Year	Protein accession nos.
<i>F. tularensis</i> subsp. <i>holartica</i> FTN002-00 (1, 24)	NC_009749	France	Human	2002	YP_001428362.1, YP_001428440.1
<i>F. tularensis</i> subsp. <i>tularensis</i> SCHU 54 (11, 30)	NC_006570	USA	Human	1941	YP_169632.1, YP_169697.1
<i>F. tularensis</i> subsp. <i>mediasiatica</i> FSC147 (29)	NC_010677	Kazakhstan	Mammal	1965	YP_001891444.1, YP_001891837.1
<i>F. tularensis</i> subsp. <i>tularensis</i> WY96-3418 (2)	NC_009257	USA	Human	1996	YP_001121984.1, YP_001122050.1
<i>F. tularensis</i> subsp. <i>novicida</i> U112 (28, 39)	NC_008601	USA	Water	1950	YP_898644.1, YP_898713.1
<i>F. tularensis</i> subsp. <i>holartica</i> OSU18 (34)	NC_008369	USA	Mammal	1978	YP_763419.1, YP_763476.1
<i>F. tularensis</i> subsp. <i>tularensis</i> FSC198 (7, 23)	NC_008245	Slovakia	Arthropod	1986	YP_666764.1, YP_666829.1
<i>F. tularensis</i> subsp. <i>holartica</i> LVS (19)	NC_007880	Russia	Unknown	1961	YP_513599.1, YP_513668.1
<i>F. tularensis</i> subsp. <i>holartica</i> URF11 (15)	NZ_ABAZ000000000	France	Human	1998	YP_06557761.1, ZP_06558201.1
<i>F. tularensis</i> subsp. <i>tularensis</i> MA00-2987 (13)	NZ_ABR100000000	USA	Human	2000	ZP_05247260.1, ZP_05247325.1
<i>F. tularensis</i> subsp. <i>tularensis</i> FSC033 (6)	NZ_AAYE000000000	USA	Mammal	Unknown	EDN34196.1, ZP_04986245
<i>F. tularensis</i> subsp. <i>holartica</i> FSC022 (19)	NZ_AAYD000000000	Japan	Human	1950	ZP_04985240.1, ZP_04985297.1
<i>F. tularensis</i> subsp. <i>holartica</i> 257 (32)	NZ_AAUD000000000	Russia	Arthropod	1949	ZP_04983583.1, ZP_04983660.1
<i>F. tularensis</i> subsp. <i>holartica</i> FSC200 (40)	NZ_AASP000000000	Sweden	Human	1998	ZP_02275110.1, ZP_02275799.1

FTU-1	-----MRLVTTLSLIPSIILAAP-----QLDDSFKNLENKYDGKIGIYTLNTDDKTNIKYNESYHFFIC SVFKFL LG
CAJ79396	-----MKKIITLIIGIIFITFSFATTKIDTQVTNDIQNIEKKHGGKIGVYTINRNDWSNFAVNASFYFPI CTYKFL VV
NMC-A	MSLNVKQSRIAILFSSCLISISFFSQAN-----TKGIDEIKNLETDFNGRIGVYALDTGSGKSFSYRANERFPL CSF KGF
KPC-2	MSL---YRRLVLLSCLSWPLAGFSATALT-----NLVAEPFAKLEQDFGSGSIGVYAMDTGSGATVSYRAERFPL CSF KGF
SME-1	MSKNVFKTASFLFSVCLALSAFNAHANKS-----DAAKQIKKLEEDFDGRIGVFAIDTGSNGTFYRSDERFPL CSF KGF
SFC-1	MSR---TGRLSVFFSAIFPLLTNTMAEASQPPQVTVDKLRLENDFGGRIGVYAITDGSNKTFGYRANERFPL CSF KGF
BIC-1	MAR---PSKLALSFSLLLPFLPFTSFAETWPQG---DIARQKIVKLEKDFGGRIGVSAIDTGANRTDFRADERFPL CSF KGF
BEL-1	-----MKLLLYPLLFLVIPAFA-----QADFEHAISDLEAHNQAKIGVALVSENGLIQGYRANERFAM CSF KFL
GES-1	-----MRFIHALLLAGIAHSAYASE-----KLTFKTDLEKLEREKAAQIGVAIVDPQGEIVAGHRMAQRFA CSF KFL

FTU-1	AAILDYMHNQGFLLDKKIPINQDDIGKLGYPITAKNVGKT-LTISQLNYAAIL- SDS PASNILVR-ELGGLQNLNKF IKKL GD
CAJ79396	GAILKQSMTDNKLNLQKIKISKNIQI--VEYSPITRRHINQ-TMTVKQLCQASMQ- GDN TATNILE-KLGGKLNLFILSLAD
NMC-A	AAVLKGSQDNRLNLNQIVNYNTRSL--EFHSPITTKYKNG-MSLGDMAAAALQ YSDN GATNIIERYIGGPEGMTKFMRSIGD
KPC-2	AAVLARSQQQAGLLDTPIRYGNAL--VPWSPISEKYLTTG-MTVAELSAAAVQ YSDN AAANLLK-ELGGPAGLTAFMRSIGD
SME-1	AAVLERVQQKLDINQVKYESRDL--EYHSPITTKYKSG-MTLGDMASAAALQ YSDN GATNIIERFLGGPEGMTKFMRSIGD
SFC-1	AAVLKSQQQEGLLNQIRYDNRVM--EPHSPVTEKQITG-MTVAELSAATLQ YSDN GAANLLLEKLIGGPEGMTSFMRSIGD
BIC-1	GAVLSHSQQQEGLEKRIDYKNRVM--EPHSPISAQHSSTG-MTVAQLAAALQ YSDN GATNLLLENVLGGPAGMTTFMRTLGD
BEL-1	ALVLSRIDAGEENPERKLHYDS AFL--EEYAPAAKRYVATGYMTVTEAIQSAQL SDN AAANLLK-EVGGPPLLTKYFRSLGD
GES-1	ALVFERIDSGTERGDRKLSYGPDMI--VEWSPATERFLASGHMTVLEAAQAAVQ LSDN GATNLLLR-EIGGPAAMTQYFRKIGD

FTU-1	NDTIITADE PEIN YTPHNSINKITPKAITKDIYKLAFG-NILDKKHDKDIFIKYLQDNNTGANRIAFGMPKDWIIGD KTGT CGQ
CAJ79396	HATKVAN LEPK VNHVSLTTNENKTPKIMARDINKLAFSDDDILDKHRLMFKQWLIASNTSNNHIAAEVPEDEWVG DKTGT C-Q
NMC-A	EDFRLDR WELE LNTAIPGDERDSTPAAVAKSLTLALG-NILSEHEKETYQTLKGNTTGAARIRASVPDWWVG DKTGS CA
KPC-2	TTFRLDR WELE LNSAIPGDARDTSSPRAVTESLQKLTG-SALAAPQRQQFVDWLKGNTTGNHRIRAAVPADWAVG DKTGT CGV
SME-1	NEFRLDR WELE LNTAIPGDKRDTSTPKAVANSLNKLALG-NVLNAKVKAIIYQNLKGNTTGDARIRASVPADWVG DKTGS CA
SFC-1	NVFLRLDR WELE LNSAIPGDDRDSTPKAVAESMQKLAFG-NVLGLTERHQLMDWFKGNTTGGARIRASVPANWVG DKTGT CGV
BIC-1	TTFRLDR WELE LNSAIPGDDRDSTPHAIARSLQKIALG-EALQTAAPRQQLVDWLIGNTTGGARIRAGVPVWVG DKTGT CGV
BEL-1	KVSRLDR IEP LTNTPTGDERDSTTPMSMAQTVSKLIFG-DTLTYKSKGQLRRLLIGNQTGDKTIRAGLPDSWVTG DKTGS C-A
GES-1	SVSRLDR KEPE MGDNTPGDLRDTTTPIAMARTVAKVLYG-GALTSTSTHTIERWLIGNQTGDATLRAGFPKDWVG KEKGT C-A

FTU-1	YAATNDVAIIWPKNQPIALGILYTNPNNDKNAPSNEEIIQQAAKLIAN--DLTNTYK
CAJ79396	YGTNDVAIIWPDNDRAVIMAFYTTQ-SQNAKPNKSKIVREVTKILLNRLQLNNTTKNA
NMC-A	YGTANDYAVVWPKNRAPLIISVYTTK-NEKEAKHEDKVIAEASRIAIDNLK
KPC-2	YGTANDYAVVWPTGRAPIVLAVYTRA-PNKDDKHSEAVIAAAARLALGLEGLGVNGQ
SME-1	YGTANDYAVIWPKNRAPLIVSIYTTT-RKSKDDKHSDKTAEASRIAQAIQ
SFC-1	YGTANDYAVIWPVGHAPIVLAVYTSK-PDKNSKHSDAVIDASRIVLESFNIDALRMATGKSIGF
BIC-1	YGTANDYAVIWPKTSAPIVLAIYTA-KPKEDKHSDAVIAEVTRAVLESFE
BEL-1	NGGRNDVAFFITTAGKKYVLSVYTNA-PELQGEERALLIASVAKLARQYVVH
GES-1	NGGRNDIGFFKAQE-RDYAVAVYTNA-PKLSAVERDELVASVGQVITQLILSTDK

FIG 1 Comparison of the amino acid sequences of putative class A carbapenemases from *Francisella tularensis* subsp. *holarctica* LVS (FTU-1 and CAJ79396) and those of known class A carbapenemases (NMC-A, KPC-2, SME-1, SFC-1, and BIC-1) and weak carbapenemases (BEL-1 and GES-1). The conserved ⁷⁰SXXX⁷³, ¹³⁰SDN¹³², ¹⁶⁶EXXXN¹⁷⁰, and ²³⁴KTG²³⁶ motifs are shown in bold. Highlighted in gray are the conserved cysteine residues, Cys69 and Cys238. The leader sequence of FTU-1, which is removed to give the mature enzyme, is underlined.

ensis subspecies (*tularensis*, *holarctica*, *novicida*, and *mediasiatica*), all encode two putative class A carbapenemases (Table 1).

The genes for the predicted β -lactamases are present on the chromosome in each *F. tularensis* strain. The G+C content of both genes is between 29 and 31% and is similar to the G+C content of the *F. tularensis* chromosome, which is 32% according to the GenBank database. The representative organisms were isolated at different time periods and from various sources in different parts of the world (Table 1), indicating that these proteins are native to *F. tularensis*.

The genes for both putative class A carbapenemases were recently cloned from one of the strains, *F. tularensis* subsp. *holarctica* LVS (accession number NC_007880), and expressed in *E. coli* (3). Antibiotic susceptibility testing by the disc diffusion assay demonstrated that one of the encoded enzymes (accession number YP_513599.1) produces resistance to penicillin antibiotics while the other is a nonfunctional β -lactamase (accession number YP_513668.1). No further characterization of this enzyme was done. The focus of our studies was on the active enzyme, which we call FTU-1 (from *Francisella tularensis*).

Comparison of FTU-1 with class A β -lactamases. Sequence analysis of the gene encoding FTU-1 reveals that the 864-bp open reading frame would express a 32.0-kDa preprotein that is predicted to be processed to a 30.1-kDa mature form with a pI of 7.1 (Fig. 1) (12, 20). It contains the conserved ⁷⁰SXXX⁷³, ¹³⁰SDN¹³², ¹⁶⁶EXXXN¹⁷⁰, and ²³⁴KTG²³⁶ motifs characteristic of class A serine β -lactamases, in addition to the two cysteine residues (Cys69 and Cys238) conserved in all class A carbapenemases (42). FTU-1 shares the amino acid sequence identity with the class A β -lactamases BES-1 (33.8%), CTX-M-38 (30.8%), VEB-1 (29.3%), SHV-1 (26.4%), TEM-1 (24.2%), and PER-1 (21.3%). Compared to other class A carbapenemases, FTU-1 shares 35.0% amino acid identity with NMC-A, 34.9% with KPC-2, 34.4% with SME-1, 32.4% with SFC-1 from *Serratia fonticola*, 32.3% with BIC-1 from *Pseudomonas fluorescens*, 29% with BEL-1 from *Pseudomonas aeruginosa*, and 26.7% with GES-1. The low amino acid identities with other class A β -lactamases, including carbapenemases, indicate that FTU-1 constitutes a new distinct branch of class A β -lactamases.

FTU-type β -lactamase genes are well conserved among the

FIG 2 Alignment of FTU-1 and its homologs in *F. tularensis* genomes. The arrows indicate the conserved Cys69 and Cys238. The positions where the amino acid sequence varies are underlined. In the following descriptions of the proteins, accession numbers are given in parentheses. (a) FTU-1 is found in *F. tularensis* subsp. *holarctica* strains LVS (CAJ79318), FSC200 (ZP_02275799.1), FTNF002-00 (YP_001428362.1), 257 (ZP_04983583.1), OSU18 (YP_763419.1), FSC022 (ZP_04985240.1), and URFT1 (ZP_06558201.1). (b) FTU-1 homolog from *F. tularensis* subsp. *mediasiatica* FSC147 (YP_001891444.1). (c) FTU-1 homolog from *F. tularensis* subsp. *tularensis* WY96-3418 (YP_001122050.1). (d) FTU-1 homolog from *F. tularensis* subsp. *tularensis* strains FSC198 (YP_666764.1), FSC033 (ZP_04986245), SCHU S4 (YP_169632.1), and MA00-2987 (ZP_05247260.1). (e) FTU-1 homolog from *F. tularensis* subsp. *novicida* U112 (YP_898713.1).

chromosomes of various *F. tularensis* subspecies. Multiple amino acid sequence alignments of FTU-1 and its homologs from the other sequenced *F. tularensis* genomes highlight their high degree of conservation (Fig. 2). In fact, they differ by only one or two amino acids, with the exception of the enzyme from *F. tularensis* subsp. *novicida*, which deviates by 16 residues. Thus, in spite of their diverse geographic and environmental distribution, these enzymes have retained high identity between themselves.

Antibiotic susceptibility testing. The MICs of selected β -lactams and their combinations with β -lactamase inhibitors against *E. coli* JM83 harboring FTU-1 are given in Table 2. FTU-1 confers resistance to penicillins, with MICs ranging from 64 to 1,024 μ g/ml. In addition, it elevates the MIC of ceftazidime 4-fold above background. Interestingly, FTU-1 fails to elevate the MIC of any other β -lactam tested, including the first- and second-generation cephalosporins. This exceptionally narrow spectrum of conferred resistance distinguishes FTU-1 from the majority of class A β -lactamases in Gram-negative bacteria that are typically capable of producing at least low-level resistance to early-generation cephalosporins. The MICs of penicillins were moderately reduced in the presence of β -lactamase inhibitors; the MIC of ticarcillin was reduced 4-fold when tested in the presence of 2 μ g/ml clavulanic acid, while the MIC of piperacillin was reduced 2-fold in the presence of 4 μ g/ml tazobactam. The high degree of sequence conservation between FTU-1 and its homologs in the chromosomes of *F. tularensis* subspecies makes one wonder whether these enzymes serve any additional function beyond providing resistance to penicillins.

Kinetic characterization of FTU-1. The enzyme was purified to apparent homogeneity, as judged by SDS-PAGE, and the steady-state kinetic parameters for hydrolysis of selected β -lactams were evaluated (Table 3). In agreement with the MIC

TABLE 2 Antimicrobial susceptibility profile of *E. coli* JM83 expressing the FTU-1 β -lactamase

Antimicrobial	MIC (μ g/ml)	
	Control ^a	FTU-1
Benzylpenicillin	32	256
Ampicillin	2	256
Ampicillin/sulbactam ^b	2	32
Amoxicillin	4	1,024
Amoxicillin/clavulanic acid ^b	2	64
Oxacillin	128	512
Ticarcillin	2	1,024
Ticarcillin/clavulanic acid ^c	2	256
Piperacillin	2	64
Piperacillin/tazobactam ^d	2	32
Cephalothin	4	4
Cefuroxime	1	1
Ceftazidime	0.125	0.5
Cefotaxime	0.031	0.031
Ceftriaxone	0.031	0.031
Cefepime	0.031	0.031
Cefoxitin	2	2
Cefmetazole	0.5	1
Moxalactam	0.25	0.25
Aztreonam	0.063	0.063
Imipenem	0.125	0.25
Meropenem	0.031	0.031
Ertapenem	≤ 0.008	≤ 0.008
Doripenem	0.031	0.031

^a Parental *E. coli* JM83 strain harboring the vector pHF016 containing no β -lactamase gene.

^b The β -lactamase inhibitor (sulbactam or clavulanic acid) was used at a ratio of 1:2 with the β -lactam.

^c Clavulanic acid was held constant at 2 μ g/ml.

^d Tazobactam was held constant at 4 μ g/ml.

TABLE 3 Substrate profile of the FTU-1 β -lactamase

β -Lactam	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\text{M}^{-1} \text{s}^{-1}$)
Ampicillin	170 ± 7	120 ± 20	$(1.4 \pm 0.2) \times 10^6$
Benzylpenicillin	270 ± 10	210 ± 20	$(1.3 \pm 0.1) \times 10^6$
Oxacillin	54 ± 5	110 ± 20	$(4.7 \pm 0.9) \times 10^5$
Cephalothin	0.10 ± 0.01	46 ± 4	$(2.3 \pm 0.2) \times 10^3$
Cefuroxime	0.010 ± 0.001	14 ± 2	$(7.1 \pm 0.1) \times 10^2$
Ceftazidime	0.022 ± 0.001	55 ± 7	$(4.0 \pm 0.6) \times 10^2$
Aztreonam	ND ^a	ND	ND
Cefoxitin	ND	ND	ND
Imipenem	0.017 ± 0.001	31 ± 4	$(5.2 \pm 0.8) \times 10^2$
Meropenem		≥ 100	$(2.5 \pm 0.3) \times 10^2$
Doripenem		≥ 100	$(2.1 \pm 0.1) \times 10^2$
Ertapenem	0.017 ± 0.001	33 ± 2	$(5.1 \pm 0.3) \times 10^2$

^a ND, not detected.

data, penicillin antibiotics were good substrates for FTU-1. Despite high K_m values ($\geq 100 \mu\text{M}$), in combination with high turnover numbers (54 to 270 s^{-1}), FTU-1 can attain catalytic efficiencies of 10^5 to $10^6 \text{ M}^{-1} \text{ s}^{-1}$ when hydrolyzing penicillins. Cephalosporins are poor substrates of FTU-1. Although the relative affinity of the enzyme for cephalosporins is greater than for penicillins, turnover is sluggish, leading to k_{cat}/K_m values in the range of 10^2 to $10^3 \text{ M}^{-1} \text{ s}^{-1}$. No hydrolysis of the monobactam aztreonam or the cephamycin cefoxitin was detected.

The purified FTU-1 β -lactamase was capable of hydrolyzing carbapenem antibiotics but with very low catalytic efficiency ($k_{\text{cat}}/K_m = 10^2 \text{ M}^{-1} \text{ s}^{-1}$). Imipenem and ertapenem had Michaelis constants in the low-micromolar range, but they were too high to be measured for meropenem and doripenem. The half-life of imipenem and ertapenem was $\sim 40 \text{ s}$, showing their stability in the presence of FTU-1. This stability provides the reason that FTU-1 cannot confer resistance to this class of β -lactams. The k_{cat} values obtained for imipenem are similar to those previously reported for weaker carbapenemases such as GES-1 (0.0059 s^{-1}) (16) but lower than those for more efficient enzymes such as GES 5 (0.44 s^{-1}) (16) or KPC-2 (15 s^{-1}) (43). The relative affinity for imipenem was similar to that of KPC-2 ($K_m = 51 \mu\text{M}$) (43) but lower than that of GES-1 ($K_m = 1.9 \mu\text{M}$) or GES-5 ($K_m = 1.5 \mu\text{M}$) (16).

We evaluated the inhibitor dissociation constants for β -lactamase inhibitors with FTU-1. Tazobactam had the highest affinity for FTU-1 ($K_i = 300 \pm 50 \mu\text{M}$), while clavulanic acid and sulbactam affinity was in the low-millimolar range ($K_i = 1.0 \pm 0.2 \text{ mM}$ and $2.3 \pm 0.7 \text{ mM}$, respectively). FTU-1 has less affinity for clavulanic acid than other class A carbapenemases, such as GES-2 ($K_i = 5.0 \mu\text{M}$) (18) or KPC-2 ($K_i = 1.5 \mu\text{M}$) (43). This trend is also observed for sulbactam and tazobactam with KPC-2 ($K_i = 135$ and $78.5 \mu\text{M}$, respectively) (33) and for tazobactam with GES-2 (700 nM) (17).

Conclusion. Here, we describe a chromosomal class A β -lactamase FTU-1 from *F. tularensis* subsp. *holarctica* LVS. The enzyme contains the conserved motifs and cysteine residues characteristic of class A carbapenemases; however, the antibiotic resistance profile and steady-state kinetic parameters indicate that FTU-1 is a non-extended-spectrum β -lactamase that possesses low carbapenemase activity, resulting in a 2-fold increase in the MIC of imipenem. FTU-1 shares 94 to 100% amino acid sequence identity with its homologs encoded in the chromosome of all *F. tularensis* isolates whose genomes have been sequenced. Thus,

FTU-1 is the first class A β -lactamase with carbapenemase activity that is native to *F. tularensis*.

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