Genome of a chronic osteitis-causing *Clostridium tetani*

P.-E. Fournier[1,2], P.-Y. Levy[1,2], M. Million[1,3], O. Croce[1], C. Blanc-Tailleur[1], P. Brouqui[1,3] and D. Raoult[1,2]

1) *Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UM 63, CNRS 7278, IRD 198, INSERM U1095, Institut Hospitalo-Universitaire Méditerranée Infection, Faculté de Médecine, Aix-Marseille Université, 27 Boulevard Jean Moulin,* 2) *Fédération de Microbiologie, Centre Hospitalier Universitaire de La Timone, 264 Rue Saint Pierre, 13005 and* 3) *Service de Maladies Infectieuses, Hôpital Nord, AP-HM, Chemin des Bourrely, 13013, Marseille, France*

Abstract

We sequenced the genome of a *Clostridium tetani* strain that caused chronic tibial osteitis without any clinical sign of tetanus in a 26-year-old man previously vaccinated against this disease. The genome contained a plasmid that harboured the *tetX-tetR* tetanospasmin operon, and was highly similar to that of a tetanus-causing strain.

Keywords: Chronic osteitis, *Clostridium tetani*, genome, neurotoxin, tetanospasmin

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Corresponding author: P.-E. Fournier, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UM 63, CNRS 7278, IRD 198, INSERM U1095, Institut Hospitalo-Universitaire Méditerranée Infection, Faculté de Médecine, Aix-Marseille Université, 27 Boulevard Jean Moulin, 13005, Marseille, France.
E-mail: pierre-edouard.fournier@univ-amu.fr

In August 2011, a 26-year-old man, vaccinated against tetanus in 1997, was admitted to hospital in Marseille, France, for an open fracture of his left tibia, contaminated with soil (P. Y. Levy, unpublished data). He had specific anti-toxin antibodies as measured using the immunochromatic test (Tetanos Quick Stick Test, InGen, Chilly-Mazarin, France). Due to suppuration of the wound despite surgical revision and osteosynthesis, a bone biopsy was performed, from which *Clostridium tetani* (susceptible to all tested antibiotics) was cultivated. Translucent grey colonies were obtained after 4 days of incubation in an anaerobic atmosphere on blood-enriched Columbia agar at 37°C. Production of tetanospasmin by the infecting strain was confirmed by the French reference centre for anaerobic bacteria by inoculation to mice. The patient did not develop any symptoms of tetanus. Despite several prolonged antibiotic therapies and surgical revisions, including the removal of a tibial sequestrum, as well as the administration of a dose of tetanus vaccine and prophylactic immunoglobulins, no tibial consolidation could ever be obtained. Seventeen months after the accident, the patient underwent amputation of his left leg. In order to investigate the virulence factors carried by the patient's strain, notably the presence of a toxin, we sequenced its genome.

Following DNA extraction from the patient's strain (strain 12124569) using the phenol–chloroform method, paired-end sequencing was performed using a 454 GS/FLX pyrosequencer (Roche Diagnostics, Meylan, France). The sequencing generated 792 841 reads, for a total output of 142 Mb. Following assembly, strain 12124569 had a chromosome size of 2 807 481 bp and exhibited a single 58 369 bp plasmid (GenBank accession numbers HG530135 and HG530136, respectively). The assembly was performed using Newbler 2.8 software (Roche). Finishing was achieved using the CLC genomics software (http://www.clcbio.com/). Open reading frames (ORFs) were predicted using Prodigal (http://prodigal.ornl.gov). The predicted bacterial protein sequences were searched against the GenBank database and the Clusters of Orthologous Groups (COG) databases using BLASTP. The tRNAScanSE tool [1] was used to find tRNA genes, whereas ribosomal RNAs were found by using RNAmmer [2] and BLASTn against GenBank.

Prior to our study, only one *C. tetani* genome, from toxigenic strain E88, had been sequenced [3]. By comparison with strain E88 (GenBank accession number NC_004557), strain 12124569 had a chromosome of a similar size (2.8 Mb) but a smaller plasmid (58 vs. 74 kb, respectively). Both chromosomes were highly collinear but strain 12124569 had a slightly higher GC content (28.7% vs. 28.6%, respectively). Strain 12124569 had 2845 predicted protein-coding genes, five rRNA operons, 52 tRNAs, one tmRNA, and 66 miscellaneous other RNAs. Both *C. tetani* strains exhibited very similar gene contents, and each notably had the plasmidic tetanospasmin-coding gene *tetX* as well as its transcriptional regulator *tetR* [4]. Both *tetX* genes were highly similar except a few synonymous single-nucleotide polymorphisms (SNPs). In addition, both plasmids also carried the *colT* gene that predictably codes a collagenase involved in tissue lysis [3].

Clostridium tetani is an environmental bacterium that causes tetanus, a toxi-infection associated with a high mortality. Most

of its pathogenesis results from the neurotoxin, also named tetanospasmin that it produces within the body once spores have contaminated a wound. In France, tetanus is a reportable but rare disease because of compulsory vaccination (French vaccine calendar 2012: http://www.sante.gouv.fr/calendrier-vaccinal-detaille-2012.html). Between 2008 and 2011, only 36 cases of tetanus were reported. In addition to tetanus, rare cases of focal *C. tetani* infections have been described, including a patient with humerus osteitis that resolved using antibiotics but for whom the toxin production by the infecting strain was not evaluated [5]; and a case of wound infection without osteitis caused by a proven toxigenic strain. The patient in this case readily improved after treatment with prophylactic immunoglobulins, tetanus vaccine, and antibiotics [6]. However, the case presented here was unusual in that the patient developed a chronic *C. tetani* infection caused by a toxigenic strain that persisted despite repeated antibiotic therapies and surgical revisions, without any clinical manifestation of tetanus. However, the genomic analysis of the strain infecting the patient did not reveal any difference when compared to the toxigenic strain E88, and strain 12124569 also harboured the tetanospasmin-coding gene *tetX* and was proven to be toxin-producing. We conclude that it is likely that the vaccinal status of the patient protected him from developing tetanus but not from chronic *C. tetani* infection.

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Conflict of Interest

None declared.

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