Detection of a Possible Bioterrorism Agent, *Francisella* sp., in a Clinical Specimen by Use of Next-Generation Direct DNA Sequencing

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Deep sequencing detected a potential bioterrorism agent, *Francisella tularensis*, in a human abscess sample (Iwaki-08) of unknown etiology. Identified single-nucleotide variations suggest that the Iwaki-08 case was associated with *Francisella tularensis* subsp. *holarctica* (biovar *japonica*) but not the highly virulent type A (*Francisella tularensis* subsp. *tularensis*).

CASE REPORT

In 2008, a 57-year-old man living in the Fukushima Prefecture, Japan, visited a clinic with fever and chills. He was admitted to the surgical ward of Iwaki Kyoritsu General Hospital due to a right thumb ulcer, lymphadenopathy of the right axillary lymph node, and pain in the wrist through the right elbow joint. Clinical examination revealed the ulcer to be about 2 cm in diameter at the dorsal region of the right thumb, and mild edema with tenderness appeared through the base of the right thumb to the right wrist. He complained of discomfort around the right elbow without obvious edema. There was mild tenderness and a palpable elastic hard tumor in the right axilla, 7 cm in diameter, but no redness on the skin. Laboratory findings showed an elevated level of monocytes and a high level of C-reactive protein (CRP). Cefcapene pivoxil hydrochloride (CFPN) was orally administered (300 mg/day for 5 days) based on the diagnosis of cellulitis and lymphadenopathy. In response to this treatment, the skin ulcer on the right thumb and the sizes of the lymph nodes decreased.

One month later, he visited again, complaining of right axillary redness. The skin ulcer at the dorsal region of the right thumb was diminished (Fig. 1A), and the swelling of the axillary node with skin redness was reduced to 3 cm in diameter (Fig. 1B and C), but it still fluctuated. No other symptoms appeared except lymphadenopathy. Pus from the punctured axillary abscess was cultured, but no bacteria were detected. The patient finally explained that he had skinned a hare and a neighbor had cooked the hare meat. Streptomycin sulfate (SM) (1 g/day) was intramuscularly administrated, and minocycline hydrochloride (MC) was orally administered (200 mg/day) for 14 days. After administration of SM for 14 days, drainage had diminished. In accordance with clinical improvement, the dose of administration of MC at 200 mg/day for 14 days was decreased to 100 mg/day for 56 days.

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FIG 1 Ulcer at dorsal region of right thumb (A) and swelling of axillary node with skin redness (B and C).
Next-generation DNA sequencing allows the determination of abundant DNA sequences by deep sequencing and is a powerful technique that can identify sequences of possible pathogens in clinical specimens with an unbiased comprehensive approach (4, 6). The pus sample from the punctured axillary abscess was used for comprehensive pathogen detection using the analysis strategy shown in Fig. 2A. The deep sequencing and homology search suggest that 833 reads (0.002% of a total of 38,285,502 reads) could be identified as bacterial (Fig. 2B), and the taxonomic classification indicated that the possible pathogen was similar to Francisella sp. (Fig. 2B). Further classification based on the subspecies group revealed that those reads showed remarkable similarity to Francisella tularensis subsp. tularensis NE061598 type A.I (398 reads; 47.7% of 833 Francisella reads) and Francisella tularensis subsp. holarctica type B (196 reads; 23.5% of 833 Francisella reads) (Fig. 2B). Other
potential pathogens, including viruses, were not detected in the Iwaki-08 sample (data not shown).

_F. tularensis_ subsp. _tularensis_ causes life-threatening type A tularemia and is found only in North America (1, 3), whereas _F. tularensis_ subsp. _holarctica_ causes less-severe type B tularemia and is found throughout the Northern Hemisphere, including Japan (2, 9). To determine the subspecies and potential virulence of the detected _Francisella_ sp. from the Iwaki-08 case, we performed a phylogenetic analysis based on whole-genome single-nucleotide variations (SNVs) among _Francisella_ species. Using the obtained 833 reads, 66 SNV sites were found in the 833 _Francisella_ reads among the 3,274 SNV sites in _F. tularensis_ SCHU S4 (type A.I) reference genome sequence (Fig. 3A). Nucleotide alignment and the phylogenetic tree of 11 strains, including Iwaki-08, based on those 66 SNVs, indicate that the Iwaki-08 case is apparently grouped into the subsp. _holarctica_ and is closely related to biovar _japonica_ FSC022 (Ebina) rather than other subspecies, such as type A or B (Fig. 3B). Previous phylogeography studies have demonstrated that FSC022 is a subclade representing the collapsed Japanese _F. tularensis_ subsp. _holarctica_ and that it may constitute a separate subspecies, _japonica_ (2, 7, 9).

In this case, we could not isolate the potential pathogen from the abscess sample, presumably because of the antibiotic treatment. Generally, 16S rRNA gene sequencing is affordable for bacterial identification, but _F. tularensis_ subsp. cannot be discriminated because of the high similarity of 16S rRNA gene sequences among the subspecies. In addition, _Francisella_ genome DNA is present in very small amounts at 0.002% in the abscess sample (Fig. 2B). Thus, we expected that this approach could provide comprehensive genetic information for pathogen hunting and further molecular genotyping.

In conclusion, deep sequencing was able to identify the pathogen, and further SNV genotyping of the clinical isolate revealed that the Iwaki-08 case is likely a sporadic case of _F. tularensis_ subsp. _holarctica_ (biovar _japonica_) infection in a local area of Japan rather than the result of a bioterrorism attack. It contributes to biosecurity by allowing the identification of pathogens, and the following strain-specific genotyping allows prompt traceability.

**Nucleotide sequence accession number.** Obtained short reads have been deposited in the DDBJ Sequence Read Archive (DRA) of Japan (accession number [http://www.ncbi.nlm.nih.gov/sra/?term=DRA00044](http://www.ncbi.nlm.nih.gov/sra/?term=DRA00044)).

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The study protocol was approved by the institutional medical ethics committee of the National Institute of Infectious Diseases and Iwaki Kyoritsu Hospital in Japan. Written consent for the biopsy was obtained from the patient.

**REFERENCES**