THREAT AGENT DEFEAT MODELING AND TESTING

Isolation-free Genomic Characterization Of Biological Threat Agents From Complex Samples

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Background: Many traditional biological threat agents occur naturally in the environment in regions of the world where Warfighters operate and/or testing and simulation activities are conducted. Genomic characterization can be useful for characterizing the natural background of these agents in a given operational or testing environment, and that background information can subsequently be used to distinguish between natural background and biowarfare or testing events. However, genomic characterization is hampered by the fact that many biological threat agents can be difficult or impossible to isolate from complex samples, such as aerosols or other environmental samples, as well as human clinical samples obtained from Warfighters after the administration of medical countermeasures. Metagenomics – sequencing all of the DNA present in a complex sample – is possible, but results in limited information for the targeted biological threat due to the overwhelming background present in complex samples. Targeted DNA capture and enrichment offers an alternative approach.

Purpose, objective, and rationale of the research: To demonstrate, using Francisella tularensis as a model, that targeted DNA capture and enrichment approaches can be used to genomically characterize biological threat agents present in complex samples in the absence of isolates.

Methods: RNA capture probes were designed based upon the known pan genome of F. tularensis and other diverse species in the family Francisellaceae. Probes that targeted genomic regions also present in non-Francisellaceae species were removed, and probes specific to particular Francisella species or phylogenetic clades were identified. The capture-enrichment system was then applied to diverse complex DNA extracts containing low-level Francisella DNA, including human clinical tularemia samples and environmental samples (i.e., animal tissue and air filters), which was followed by sequencing of the enriched samples. The enrichment results for the clinical tularemia samples were compared to metagenomics results for the same samples.

Results and conclusions: Analysis of the resulting data facilitated rigorous and unambiguous confirmation or rejection of the detection of F. tularensis and/or other Francisella species in complex samples, identification of mixtures of different Francisella species in the same sample, analysis of gene content (e.g., known virulence and antimicrobial resistance loci), and high-resolution whole genome-based genotyping that could be used for source attribution. Deep metagenomic sequencing of the clinical samples yielded very limited information, whereas enrichment provided almost complete genomic coverage of these samples. Although not explored in this study, enrichment systems could also be constructed to capture known signatures of genetic engineering.

Impact to the DTRA mission and warfighter: Targeted DNA capture and enrichment provides a robust means to genomically characterize biological threat agents present in complex samples from which isolates cannot be obtained. This approach represents a novel approach for genomically characterizing biological threat agents in both test and operational environments, including informing use of medical countermeasures based upon characterization of antimicrobial resistance loci and geographic source attribution to distinguish between natural occurrence and biological weapons of mass destruction events.

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