Obstacles and Possibilities in Pathogen Agnostic Sequencing for Public Health Monitoring: Insights from the Worldwide Emerging Infections Monitoring Program

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Abstract

Given its use in infectious disease monitoring and detection, pathogen agnostic sequencing has been recognized as a biodefense and public health tool. Since Sanger sequencing, next-generation sequencing (NGS) technologies higher throughput genome sequencing methods that can be used for pathogen agnostic sequencing—have become more accessible and widely used in public health and biomedical labs. Additionally, the price per genome or sample sequenced has dropped significantly, hastening the shift of NGS from a research endeavor to a necessary part of routine public health monitoring. Conclusion:The GEIS program has made investments in surveillance and advanced characterization infrastructure in accordance with the biosurveillance landscape. Over time, the DOD has maintained and expanded these capabilities for broader public health surveillance, and these investments have continued to support the need for timely information on emerging infectious threats. In our opinion, these technologies will have a greater long-term impact when used in a strong and well- coordinated laboratory network for infectious disease response and surveillance.

Keywords: Public Health, Public Health Monitoring, Infections Monitoring, Pathogen.

1. Introduction

Pathogen agnostic sequencing, also known as metagenomic, shotgun, unbiased, or random-priming sequencing, is typically used when more information is desired about the microbial community within a sample or when more specific assays, such as polymerase chain reaction or amplicon sequencing, fail. These assays may fail due to suboptimal design or selection, poor or missing reference genomes, the evolution of target pathogens, or the emergence of a novel or diverge (McCombie, 2019).

Recent techniques to pathogen agnostic sequencing have concentrated on clinical metagenomics, which is utilized largely for patient diagnoses, the discovery of novel or unexpected human pathogens, and environmental sample screening. Some of the first achievements in routine pathogen agnostic sequencing were performed in the field of clinical metagenomics to find unknown pathogens in patient samples and pinpoint the likely cause of underlying disease or death.6 Pathogen agnostic sequencing methods have long been utilized in public health surveillance to detect novel and emerging animal and human diseases with the potential for spillover or pandemic (Schlaberg, 2017).

Surveillance (GEIS) program of the US Department of Defense (DOD). Given widespread access to sequencing technologies, long- standing surveillance studies with ongoing sample collections and repositories, and DOD scientists' strong expertise in NGS and bioinformatics, the GEIS program is well positioned to implement, maintain, and generate meaningful data from pathogen-agnostic sequencing within its surveillance network. We believe the DOD GEIS program has learned numerous valuable lessons about the operationalization and implementation of pathogen-agnostic sequencing inside an existing infectious disease surveillance program. The goal of this commentary is to use an existing DOD infectious disease surveillance program to highlight opportunities and limitations for implementing pathogen agnostic sequencing in the larger global community.

The Evolution of Pathogen Agnostic Sequencing in the GEIS Program:

The GEIS program is run by a global network of US Army, Navy, and Air Force medical research, clinical, and public health laboratory partners in strategic locations who are on the front lines of global infectious disease surveillance and have long-standing relationships with US interagency, host-nation, and international partners. These partners contribute laboratory-confirmed infectious disease surveillance data across the network, some of which could indicate increasing hazards to US service members. Strong sentinel site initiatives and structured, coordinated data gathering systems may also make epidemiological and clinical metadata available. This helps to complete the picture of an infectious disease pathogen by linking genesis, transmission, and clinical effects to genetic characterisation. However, this reporting does not occur in real time. While GEIS-funded partners may support speedy communications on the ground with local groups as needed, they must send data to the GEIS program office at least once a month. When high- or moderate-level threats to force health protection are discovered, partners must report back to the GEIS program office and geographic combatant commands within 24 hours to inform their decision-making (Gardy , 2018).

The GEIS initiative used early DOD biodefense and medical research expenditures in pathogen sequencing, undertaken in 2014-2015, to improve infectious disease monitoring efforts carried out by GEIS partner facilities. Some of these critical investments included the Global Biosurveillance Technology Initiative, which was run by the Joint Program Executive Office for Chemical and Biological Defense (now the Joint Program Executive Office for Chemical, Biological, Radiological, and Nuclear Defense) and provided DOD laboratories with Illumina MiSeq instruments, ancillary library preparation equipment, and bioinformatics servers. These initiatives were carried out in response to priorities identified in the 2012 National Strategy for Biosurveillance, which included the establishment of a coordinated, integrated biosurveillance infrastructure (Miller, 2013).

Pathogen agnostic sequencing in the GEIS network: Historically, the GEIS program has mostly sponsored pathogen- agnostic sequencing initiatives for surveillance programs in its respiratory, febrile, and vector-borne infection portfolios. Prior to the COVID-19 pandemic, the GEIS program targeted "pathogen-negative" samples from normal surveillance activities for agnostic sequencing, with an emphasis on acute respiratory infections or acute febrile diseases that did not respond to standard diagnoses. Pathogen agnostic approaches have also been utilized to characterize arboviruses and find pathogens in vector samples.Although not precisely "agnostic," GEIS partner laboratories have created or employed sequencing technologies that improve on existing metagenomic methodologies by incorporating optimization phases for host depletion or viral pathogen enrichment. Pathogen agnostic or panviral sequencing methods were employed in early outbreak or pandemic reactions to characterize Zika virus, SARS-CoV-2, and mpox cases within the GEIS network, before numerous reference genomes were available or standard amplicon-based sequencing protocols became widely accepted (Blazes , 2013).

Challenges in Operating a Pathogen-Agnostic Surveillance System:

1. Identifying acceptable use cases.
2. Rapidly evolving or changing sequencing and bioinformatics technologies/methods.
3. A lack of community standards.
4. Reproducibility of Results
5. Development of data and sample-sharing agreements with partner laboratories and host countries
6. Staff recruitment, retention, and training
7. Assistance with supply chain and equipment servicing, especially for low- or middle-income countries.
8. Access to computing resources, IT personnel assistance, and up-to-date databases.
9. Access to consistent financial sources for public health surveillance initiatives.
10. Integration with current clinical or public health surveillance systems.

11. Policy gaps for using pathogen-agnostic sequencing results for public health action.

Opportunities for GEIS to Use Pathogen-Agnostic Sequencing: The GEIS program has numerous chances to further enhance and apply pathogen agnostic sequencing:

1. Use and standardize testing algorithms that are not dependent on

prior (biased) focused or panel molecular assays.

2. promote novel pathogen identification across unique sample sets (e.g., environmental, vector, human) gathered by GEIS partners and promote the development of new molecular assays.

3. Provide additional information about "pathogen-negative" samples from acute febrile sickness and acute respiratory illness cases discovered by GEIS surveillance activities.

4. Acquire more USG funding streams while remaining aligned with national and international biosurveillance and biodefense priorities

5. Maintain and expand early pandemic preparedness for emerging viruses inside the US Department of Defense and among host-nation partners.

6. Work with and contribute to US government interagency and worldwide pathogen-agnostic sequencing surveillance systems.

2. Recommendations:

Maintaining a laboratory network capable and prepared to apply pathogen-agnostic sequencing technologies to identify and characterize new pathogens is difficult. Capital investments must be examined on a constant basis to guarantee that DOD laboratories remain up to date with current equipment. Furthermore, nothing is more important than keeping an outstanding staff in a fiercely competitive talent market. The COVID-19 pandemic has heightened the need for public health professionals and scientists to create detailed strategies to implement a coordinated response to risks discovered by a pathogen-agnostic surveillance system.

The GEIS mission is to preserve force health, and continuing investment in pathogen-agnostic technologies provides a chance to improve the data products offered by the GEIS partner network. Regardless of where an emerging pathogen originates, the GEIS program must continue to work with partner laboratories to guarantee and maintain a robust capability for detecting and responding to infectious disease risks.

3. Conclusion:

Previous DOD medical research and biodefense investments in NGS and pathogen agnostic testing have improved public health surveillance and prepared the way for capabilities that will undoubtedly be crucial in preparing for and responding to the next pandemic. Since its inception in 1998, The GEIS program analyzed the biosurveillance landscape and made appropriate

investments in surveillance and advanced characterisation infrastructure. These investments have supported the DOD's requirement for timely information on emerging infectious hazards over time, allowing it to sustain and enhance such capabilities for broader public health surveillance. We believe that these technologies will have a greater long-term impact when integrated into a comprehensive and coordinated laboratory network for infectious disease surveillance and response. Even though the GEIS initiative is still integrating pathogen-agnostic sequencing into partner labs, there is a basis for quickly scaling up capabilities to address new infectious disease threats and enhance public health and US military health protection. To protect against new dangers in the future, it will be essential to keep investing in these capabilities, from manpower and training to next-generation technologies.

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