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Article: In-Depth *Outbreak Tracking, Infectious Disease Diagnostics Becoming Key Applications for NGS* has been updated.

Outbreak Tracking, Infectious Disease Diagnostics Becoming Key Applications for NGS

Dec 28, 2015 | Monica Heger

Premium

NEW YORK (GenomeWeb) – In 2011, experts predicted that within five to 10 years, clinical microbiology labs would routinely sequence microbial genomes on a next-generation sequencing instrument, and that it would cost less than €100 ($109) to generate a complete bacterial genome.

Four years later, those predictions are starting to bear fruit.

"NGS is becoming more and more routine in clinical microbiology labs," Ted Pak, a graduate student in Andrew Kasarkis' lab at the Icahn School of Medicine at Mount Sinai, told GenomeWeb. His lab recently sequenced serial isolates of a patient with Stenotrophomonas maltophilia infection in order to determine whether the hospital's recent uptick in infections was an outbreak or separate transmission events.

NGS offers a level of granularity that other technologies, like PCR or traditional strain typing methods like pulsed-field gel electrophoresis, do not. It is more comprehensive than PCR, able to analyze not just known genomic sites but the entire genome. And it has a much higher resolution than pulsed-field gel electrophoresis, which cannot say whether two hospital patients infected with the same bug acquired their infections separately, or whether their infections are related — a key difference that can impact measures the hospital takes to control the spread of infection.

In the US, there are a number of signs that point to the adoption of the technology within hospitals. The US Centers for Disease Control and Prevention, for example, has set aside $2.3 million in fiscal year 2016 to roll out NGS and bioinformatics technologies for the detection of infectious disease outbreaks in

certain states.

The CDC has also been actively involved in a number of research projects. For instance, it collaborated with the Translational Genomics Research Institute to sequence multidrug resistant *Klebsiella pneumoniae* strains that produce carbapenemase enzyme, which confers resistance to the antibiotic carbapenem. These so-called KPC-producing organisms are fortunately still rare, although Brandi Limbago, deputy director of the CDC's Division of Healthcare and Quality Promotion's Clinical and Environmental Microbiology Branch, previously referred to them as "nightmare bacteria." By focusing efforts to study the mechanism by which they've acquired drug resistance now, the hope is to stem the spread of these strains before they become problematic.

The CDC is also involved with a large-scale project to sequence the genomes of 100,000 foodborne pathogens. The University of California, Davis is spearheading the 100K Genomes Project in order to build up a public database of genomes to help identify genes associated with antibiotic resistance, persistence, and pathogenesis, as well as genes that provide information about the strain's location, serotype, and its associated host.

The National Institutes of Health is also playing a significant role. Its interest in using the technology to sequence pathogens responsible for infections and hospital outbreaks was piqued in 2012, when NIH researchers used NGS to figure out how 18 patients at the NIH Clinical Center became infected with drug-resistant *K. pneumoniae*.

Comparing the genomes from all 18 patients helped determine a likely transmission route in which the first patient transmitted the bacteria to other patients on two different occasions. The sequencing results were even able to determine that transmission occurred from two different parts of her body.

Now, NIH researchers are testing whole-genome and amplicon sequencing-based protocols to diagnose microbial infections. Initially, they plan to test the methods on discarded portions of 250 samples that have received standard testing in the clinical microbiology lab in order to evaluate the utility of NGS for infectious disease diagnostics in the context of a routine hospital clinical microbiology lab, Karen Frank, chief of the microbiology service at the NIH Clinical Center, told GenomeWeb.

"Initial results are very promising," she said in an email, and the team has now progressed to testing primary specimens. Nonetheless, she said, there are still a number of challenges for integrating NGS into clinical microbiology labs, including the "expertise required for data analysis and interpretation, the absence of commercially available push-button diagnostic systems, and regulatory issues relating to validation, quality control, reporting to the medical record, and genetic privacy."

Modern microbiology

Across the Atlantic, researchers and public health officials in the UK are taking a systematic approach to moving NGS into clinical microbiology labs. For the last several years, members of the UK Clinical Research Collaboration have been sequencing pathogen genomes to build up genomic databases of various bugs. Recently, a group led by the University of Cambridge published a database of over 1,000 methicillin-resistant *Staphylococcus aureus* genomes. The genomes came from 46 laboratories that had submitted clinical isolates to the British Society for Antimicrobial Chemotherapy. The database can now

be used as a resource for future surveillance and outbreak investigations of MRSA, the authors wrote.

Aside from MRSA, the research team is building up databases of other pathogens, including vancomycin-resistant *Streptococci*, Sharon Peacock, a clinical microbiology professor at the University of Cambridge, previously told GenomeWeb.

Other researchers within the Modernising Medical Microbiology group at the University of Oxford are working on developing an NGS-based test for *Mycobacterium tuberculosis*. The group published a validation of the test in The Lancet this month, demonstrating that it was accurate, faster than traditional testing methods, and would cost about 7 percent less than current diagnostic tests.

Public Health England is now conducting a feasibility study on 2,000 samples to see if the test can be implemented in routine diagnostics, and the Modernising Medical Microbiology team is working on developing NGS-based tests for *Escherichia coli* and *S. aureus*.

Real-time tracking

Last summer, researchers put NGS technology to the ultimate test, deploying it in the midst of an ongoing outbreak of a rapidly spreading pathogen in mostly rural areas of Western Africa without major medical laboratory infrastructure.

In the midst of the Ebola outbreak, researchers with the Liberian Institute for Biomedical Research and the US Army Medical Research of Infectious Diseases (USAMRIID) set up a genome sequencing laboratory to help manage outbreaks of the virus.

Earlier this month, the team published whole-genome sequencing results of 140 Ebola isolates collected during the second wave of the outbreak in Liberia. The data indicated that most Liberian infections came from an isolate from Sierra Leone. The researchers also noted that the results suggested the second wave of the outbreak may have been different from the first wave, which began in March 2014, but fizzled out much more quickly than the second wave that began in May 2014.

Understanding the origin of an outbreak and how it spreads is important for figuring out how to manage and stem transmission, and could help better manage future outbreaks.

Some groups are pushing to implement newer nanopore sequencing technology that could generate whole-genome sequence data faster and in the field. Two groups from Europe and the US took the USB stick-sized MinIon from Oxford Nanopore Technologies to Guinea and Liberia to sequence Ebola isolates. Joshua Quick, a graduate student in Nick Loman's lab at the University of Birmingham, set up three MinIon devices, a PCR machine, and four laptops at a Guinean hospital with no lab equipment aside from a refrigerator, freezer, and a back-up generator. Quick was able to generate Ebola sequence data from samples shipped in from sites just a few hours north of the hospital, and analyzed over a dozen samples in a couple of weeks.

Challenges

Despite the progress made this year, a number of challenges remain. First, although the MinIon is especially promising for tracking outbreaks in resource-poor areas in real time, the technology is still

new and needs further validation before it can be routinely used.

Perhaps a more crucial hurdle will be to ensure that the vast amounts of pathogen sequence data are publicly available, according to Andrew Kasarskis, co-director of the Icahn Institute for Genomics and Multiscale Biology at Mount Sinai Hospital. His team is now sequencing clinical isolates of *Clostridium difficile* infections in attempt to develop a workflow for tracking infection in the hospital.

One problem with clinical data is that it is proprietary and often contains sensitive patient information, he said. But, if the hospitals and research groups work in isolation, the real insights into how pathogens spread and get transmitted, what types of antibiotic resistance elements are becoming more common, and detecting the start of an outbreak early will not be possible, he said.

For instance, if a patient with carbapenem-resistant Enterobacteriaceae was previously hospitalized in one state, but then a patient a different state becomes sick with the same strain, "you can say you've imported the bug from here to there," he said. "But if those groups keep their data siloed, you wouldn't know."

Aside from data sharing, another challenge will be managing the sheer amount of data that is produced. Hospitals and clinical microbiology labs will "have to get used to the generation, storage, and interpretation of all that data," Kasarskis said. "It's not simple."

These challenges are starting to be overcome though, Kasarskis said, as more and more clinical microbiology labs start testing NGS protocols. He predicted that such testing will be done both in large centralized laboratories as well as in hospitals.

Pak added that although PCR is a promising technology for rapid diagnostics, it "won't give you as much information as sequencing."

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