

Hospitals Build Case for Pathogen Sequencing to Track Resistance, Monitor Outbreaks

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SAN FRANCISCO (GenomeWeb) – Over the last several years, an increasing number of hospitals have begun sequencing pathogens isolated from patients who developed antibiotic resistance or whose infections were suspected to be part of an outbreak.

Many of these efforts have been [led by](#) groups in the UK, who have demonstrated the benefits of sequencing for outbreak surveillance and [predicting antibiotic susceptibility](#) or resistance, developed [NGS-based diagnostics](#) for tuberculosis and other infectious diseases, and built up [extensive databases](#) of clinical pathogen genomes.

More recently, a number of other groups in Europe and the US have also started implementing these techniques.

For instance, researchers at the University Hospital Münster in Germany have instituted a protocol to sequence pathogen genomes isolated from patients with a drug-resistant infection or who are suspected of being part of an outbreak. This has enabled them to more cost-effectively identify potential outbreaks earlier on or to rule out suspected patient-to-patient transmission.

Dag Harmsen, a microbiologist at the University of Münster, said that the clinical lab at the university hospital's Hygiene Institute currently sequences between 1,500 and 1,800 patient-derived strains each year. His team was one of the first to use sequencing in the [context of an outbreak](#), specifically the 2011 *Escherichia coli* outbreak in Europe that killed more than 50 people.

Harmsen said that the lab recently decided to switch from an Illumina instrument to using Pacific Biosciences technology in order to generate complete genome assemblies, which he said allow for better identification of antibiotic resistance genes. In addition, he expects that upcoming upgrades to the PacBio system will reduce turnaround time from several days to one day.

With the longer reads of the Sequel, Harmsen said, it is possible to generate complete genomes, including mobile elements and plasmids, making it possible to determine how antibiotic resistance genes are being transmitted. Currently, it's possible to identify the resistance genes using short-read sequencing, he said, but not to determine whether resistance is being transferred horizontally via plasmids or vertically on chromosomes.

Over the next year, the clinical team plans to validate the protocol on the Sequel and to reduce turnaround time. Currently, turnaround time is between three and four days, but Harmsen said that will be reduced to initially two days, and eventually 24 hours.

Already, the team has made some adjustments to reduce the time for the sample prep process. For instance, strains were initially grown in culture overnight before sequencing, which was necessary to obtain enough high-quality DNA. But as sequencing technology improved, it became possible to sequence DNA of lesser quality. His group settled on a

protocol that involves extracting DNA from single colonies grown on a plate, as opposed to liquid culture, which saved around 12 hours.

In the US, researchers who are part of the Pathogen Surveillance Program at the Icahn School of Medicine at Mount Sinai have expanded on a [pilot study](#) to sequence patients infected with *Clostridium difficile* and have now launched a number of longitudinal studies that focus on using sequencing to study and monitor infection, drug resistance, and transmission.

In one study focused on *C. difficile*, researchers are prospectively collecting samples from patients admitted to the intensive care unit before they have an infection. For patients who develop a *C. difficile* infection, the team then goes back and sequences the pre-infection samples, as well as the isolate.

Harm van Bakel, assistant professor at the Icahn Institute for Genomics and Multiscale Biology, said that so far, the team has sequenced samples from 325 infected patients along with around 600 controls. Ultimately, he said, the aim is to sequence up to 1,000 patients plus 2,000 controls.

The group is sequencing the microbiome of each patient using predominantly a 16S sequencing strategy on Illumina platforms, but van Bakel said they also plan to do some metagenome sequencing for cases and controls and for a limited number of samples. For this, they will use PacBio technology to get a "more complete reconstruction of individual genomes in the microbiome," van Bakel said. In addition, the researchers will sequence the genome of the *C. difficile* pathogen.

Van Bakel said that thus far, the sequencing results have provided some valuable information. For instance, about half of the patients who are admitted to the ICU already are carriers of *C. diff*, even though they do not have symptoms. "Many of these patients have long histories of hospital admissions," often with clinics outside of the Mt. Sinai health system, van Bakel said.

That's important, he noted, because it means that no matter how much effort staff put into eliminating potential sources of transmission in the ICU itself, it won't be able to solve the problem, since so many patients are entering the ICU already colonized with *C. diff*.

Andrew Kasarkis, professor of genetics and genomic sciences at Mt. Sinai, noted that this result underscores the importance of not working and treating patients in a silo. He said it's becoming clear that knowing patients' prior interactions with various healthcare facilities will be important for population health management and infection control.

Another interesting finding is that in many patients who do develop an infection, even those who are not prior carriers of *C. diff*, the researchers are detecting *C. diff* in samples three to four days prior to the onset of symptoms, van Bakel said.

Longer term, the group aims to study whether there are signatures within the microbiome that can help identify patients who are at higher risk of infection.

It has also set up a broad surveillance program, van Bakel said. As part of that, anytime a lab gets back a positive culture result for a bacterial infection, the electronic medical record automatically issues an alert. For this study, the team is currently sequencing isolates from all cases of infection with methicillin-resistant *Staphylococcus aureus*.

Like the German group, the team is using PacBio's technology to sequence the pathogen genomes "because we get complete genomes and it lets us look at accessory parts of the genome, like the gain or loss of certain elements," Kasarkis said, such as mobile elements

or plasmids that may play a role in the infection, resistance, or can help figure out transmission routes.

Currently, the team is not yet using sequencing to inform management or treatment of patients, Kasarkis said. "The turnaround time is not yet fast enough to be a routine part of the clinical infection workup for each patient," he said. That's largely because the group is still doing most of the pathogen sequencing on the older version of PacBio's technology, the RSII, he added.

However, the group does "active surveillance for outbreaks and assists in the management of any outbreak that's suspected or detected," Kasarkis said.

Real-time clinical management of patients could potentially be done when the team migrates its workflow to the Sequel, he added, which it does plan to do in the near term.

Ted Pak, an MD/PhD student in Kasarkis' lab, said that in addition to moving the workflow to the Sequel, the team would also need to "generate the evidence of the kinds of interventions you can do based on genomic sequencing."

In Germany, Harmsen's group has already started using sequencing results to inform patient management. He noted that one of the biggest benefits has been in ruling out potential cases of patient-to-patient transmission, which the [researchers estimated](#) saved the hospital €200,000 per year.

Kasarkis said that the Mt. Sinai team has not yet done a detailed economic analysis of the costs and benefits of sequencing. "Demonstrating improved patient safety is a little tricky because you have to talk about the number of infections prevented," which is difficult to do, he said. However, the team has estimated that it's possible to use electronic medical record information retrospectively to determine that patients who contracted a hospital-acquired *C. diff* infection spent about three extra days in the hospital.