A Tactical Approach To Eliminating Deadly Bacteria

Researchers are using the MiSeq® System to identify strains of drug-resistant bacteria from around the globe.

Introduction

Antimicrobial resistance is one of the global health care community's most pressing problems.[1] When penicillin was first discovered in 1928, scientists hailed it as a miracle drug that turned life-threatening infections into easily treatable conditions. By the 1940s, penicillin and other antibiotics were in wide use, saving thousands of lives worldwide. As the decades passed, antibiotics were prescribed increasingly for any condition resembling a bacterial infection. The overprescription in man and agriculture, together with the misuse of leftover antibiotics, changed the playing field in the fight against virulent bacteria.[2] Antimicrobial resistant strains of *Escherichia coli*, *Staphylococcus aureus*, and *Clostridium difficile* began to appear that even the most potent antibiotics were powerless against. According to the US Centers for Disease Control and Prevention (CDC), at least 2 million people become infected with drug-resistant bacteria every year in the United States, with an estimated 23,000 people dying as a result of those infections.[3] It's estimated that by 2050, antibiotic-resistant bacteria will kill a staggering 10 million people per year worldwide.[4] This number, quite shockingly, will surpass the number of deaths from cancer.[5]

One of the most dangerous antimicrobial resistant strains is methicillin-resistant *Staphylococcus aureus* (MRSA) which first appeared in 1960.[6] Initially, people were most likely to catch this drug-resistant "super bug" in hospitals. Today, new variants have spread in the general community, increasing the likelihood of infection through a break in the skin.

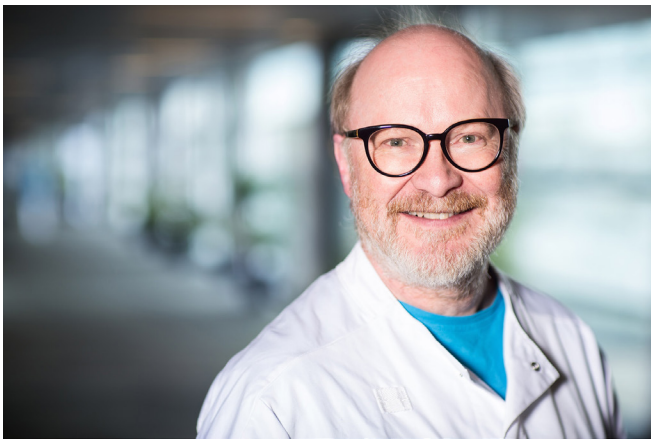
Henrik Westh, MD, Professor, PhD, a clinical microbiologist at Hvidovre University Hospital in Copenhagen, Denmark, is using next-generation sequencing (NGS) platforms, like the MiSeq System, to understand the evolution and spread of MRSA and determine the relatedness of different strains of MRSA acquired from around the world. He and a team of global collaborators believe that by understanding how this deadly bacterium spreads, they can keep MRSA at low levels within the population. They have used a technique called "*spa* typing" which involves sequencing the MRSA *spa* gene to differentiate between various strains of the bacteria. iCommunity spoke with Dr. Westh about his research and the global collaboration that Dr. Westh hopes will quash drug-resistant super bugs, saving millions of lives.

Q: What sparked your interest in clinical microbiology?
Henrik Westh (HW): I was a physician in clinical practice for 6 years and the prospect of conducting microbiology research intrigued me. It offered so many opportunities to make a difference for individual patients and for improving infection control in hospitals. It's exciting to conduct research in antimicrobial resistance and contribute to society's understanding about effective antimicrobial usage.

Q: What was sequencing technology like when you first began to use it in your studies?
HW: I began using Sanger sequencing performed with electrophoresis gel glass plates. Sequencing genomes using next-generation sequencing (NGS) technology really started around the time my first PhD student was trying to analyze a staphylococcal chromosome with long-range PCR. We couldn't get PCR to work, so we decided to spend about $10,000 to have the genome sequenced on the Roche 454 system, the only sequencer at the time. It turned out that the region she was looking at was not 10,000 base pairs long as we had estimated, but a little over 35,000 base pairs. It would have taken her months to sequence it with Sanger, but NGS offered a much faster solution. I thought, 'wow, NGS works!'

Q: When did you start incorporating NGS into your studies routinely?
HW: We began using NGS in our studies in late 2009. At the time, there were only a few places performing NGS and it was used mainly for human genome sequencing. People believed that it was better suited as a tool for research, or maybe in a reference or service laboratory. Later, we realized it could be used for microbial genomes and infection control.

Q: What impact have these NGS tools had on your research?
HW: NGS provides high-resolution data, enabling us to identify which organisms are part of the transmission chain and which aren't in terms of infection control. We couldn't do that with the old typing methods; we couldn't even see that we were making mistakes. NGS is a unique tool that is almost mistake-free.



Dr. Henrik Westh is a clinical microbiologist at Hvidovre University Hospital in Copenhagen, Denmark.

Q: What is MRSA?
HW: MRSA is an *S. aureus* bacteria that has become resistant to beta-lactam antibiotics. This includes members of the penicillin and cephalosporin antibiotic groups, which are the first and best choice antibiotics for treating seriously ill patients. MRSA was made by man using and misusing antibiotics. The agricultural industry has created livestock-associated MRSA as well, by using massive amounts of antibiotics especially in pig production.

MRSA has evolved quite a lot in the past 15 years. In the 1980s and 1990s, you only acquired it in hospitals. In the late 1990s, it became prevalent in the community. There are 3 main MRSA variants in the population today; hospital clones, community clones, and livestock-associated clones.

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Q: Why is typing MRSA strains important?
HW: Typing MRSA strains is important for infection control. If we have 2 cases of MRSA in the hospital, we want to know if they are related. If the strains are identified in the same hospital room, we know that they are connected. But if they are isolated from different departments, it might be the result of a transmission chain that we need to identify immediately.

Q: When did you begin focusing on MRSA?
HW: I began studying MRSA while writing my clinical microbiology thesis on *S. aureus*. At first, I was working on issues related to ordinary *S. aureus*, its antimicrobial resistance, and its spread in hospitals. We began looking at MRSA's evolution studying a large outbreak in Denmark in the 1960s, which followed an outbreak 2 years earlier in the UK and other parts of Europe. We found that about 20% of all bacteremic patients with *S. aureus* in Denmark had MRSA. And it was from a single clone.

We looked at how the epidemic evolved and the factors allowing us to control it. We saw, by pulsed-field gel electrophoresis (PFGE), that the old Danish MRSA isolates looked like some of the more modern clones from a Portuguese hospital. This led us to introduce *spa* typing.

Q: What is *spa* typing?
HW: *spa* (staphylococcus protein A) is a gene in the *S. aureus* chromosome. *spa* typing is a sequenced-based, universal tool. It has been very valuable in determining whether patients have transmitted their bacteria between each other. When I started, there were probably less than 200 known variants. Using *spa* typing, the scientific community has identified 15,000 variants to date. Researchers can now access the SpaServer (Seqnet.org), a fantastic international database that was created by the initiatives of Drs. Dag Harmsen and Alexander Friedrich in Germany and today contains more than 330,000 isolates.[7]

When I became a physician, there were fewer than 100 patients a year in Denmark that had MRSA. In 2003, I could see this curve changing, so we introduced *spa* typing for all our MRSA isolates. We suggested that the National Health Board of Denmark revise the country-wide MRSA guidelines, and ended up with new national guidelines in 2006. We have many rules about how to 'search and destroy' MRSA. We're trying to make sure it doesn't spread in the community, or take up residence in our hospitals and thereby infect patients.

Q: How is the *spa* gene used to type MRSA strains?
HW: *spa* typing is the most reproducible and user-friendly typing method. The *spa* sequence has several repeats, usually spanning about 24 base pairs each, that are repeated between 1–25 times within the gene. Each repeat can be different and there are many (+680) distinct types of repeats. Every time a unique sequence is identified, the *spa* type is given a number and is added to the SpaServer database.

Q: How did the *spa* typing data from the MiSeq System and Sanger sequencing compare?
HW: With the introduction of routine WGS of MRSA, we needed to ensure backward compatibility with our old sequence-based typing method. In 2014, we published data that compared *spa* typing using the MiSeq System and Sanger sequencing.[8] There was over 97% total agreement between the data sets. We're so satisfied with the MiSeq System data quality that we don't perform *spa* typing with Sanger sequencing anymore.

The speed of the 2 methods is about the same, but instead of just getting the *spa* type, the MiSeq System delivers data on the whole genome with the same turnaround time. That gives us more data for infection control and for follow-on research projects. In particular, single nucleotide polymorphism (SNP) tree analysis has shown us that SNP trees give resolution even within a *spa* type.[9]

We now test 24 genomes in a single run and run the MiSeq System 3 times a week. As Illumina NGS technology continues to improve, we're looking forward to even faster turnaround time and the ability to support automated library prep.

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Q: Is whole-genome sequencing (WGS) performed on all MRSA isolates at Hvidovre Hospital?
HW: In 2012, we began performing WGS on the MiSeq System for *spa* typing of all MRSA isolates at the hospital. We're also the regional reference laboratory, performing *spa* typing for all hospitals in the capital region.

In the beginning, I'm sure microbiologists in Denmark were thinking, 'Why is he doing this? Why is he sequencing all MRSA isolates?' I'm now seeing more microbiologists performing WGS and we already have one more microbiology department in another Danish hospital

that has bought a MiSeq System. In the next year, we intend to obtain more MiSeq Systems so that we can perform genome sequencing in all 3 facilities in our hospital network. We're also making sure that all the data ends up in a common hospital database so that we can share our information.

Q: What type of database do the *spa* typing results end up in?
HW: Results from *spa* typing, multilocus sequence typing (MLST), and Panton-Valentine leukocidin (PVL) gene presence go directly into our laboratory information system and the patient's records. This way clinicians and microbiologists know exactly what types of clones are present. With the SNP-based analysis systems that we have now, we can see that isolates we thought were identical, were very different actually. We are creating a national database built on NGS data and patient epigenetics data. International databases will become very important as we can share sequences and develop common nomenclature for identification and naming of clones.

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Q: Have you used the MiSeq System for any other microbial sequencing projects?
HW: To date, we've sequenced 4500 bacterial genomes. We've sequenced Vancomycin-resistant enterococci,[10] and several *E. coli*, especially looking for the plasmids that are responsible for beta-lactam resistance and about 10 other species. We are collaborating with colleagues at the Technical University of Copenhagen to perform metagenomic analysis. For example, we performed a study looking at the fecal microbiome to identify causes of infectious diarrhea. Routine metagenomic analysis of selected patient samples and study of viral populations will begin in 2016.

Q: When you became a clinical microbiologist, did you believe that there would be a day when WGS could be performed in 1 day?
HW: I became a microbiologist in the late 1980s and at the time it was fantastic when somebody would complete a PhD on the sequence of a plasmid, phage, or a transposon. Thinking back it's really incredible the technological strides that we've made.

Q: How has NGS transformed clinical microbiology?
HW: We have already talked about its benefits in infection control and typing. We are heading towards a better understanding of multimicroorganism infections and suitable metagenomics approaches will improve diagnosis and treatment. All the sequence data we generate will help make better PCR-based assays for faster screening. For example, we have an influenza PCR assay, but the influenza virus is a moving target. During the H1N1 epidemic several years ago, none of the PCR assays in the world were perfect. So we must make sure that our molecular assays are working perfectly every year. I think NGS will improve the quality and quality control of all these molecular methods.

> "There's a lot of evolutionary history of microbial-resistant strains that we can track with NGS."

Q: How will the declining cost of sequencing benefit clinical microbiology research?
HW: Low-cost sequencing will benefit research and infection control efforts. In Germany, if there is an outbreak in a hospital ward, the authorities close the ward and the hospital loses money until the outbreak is controlled. With NGS, you can look at suspected outbreak isolates, identify the infection points, and reopen the ward faster. NGS is also fantastic for identifying the source of food outbreaks. Last year we had a listeria outbreak from contaminated sausages. WGS identified that all of the isolates were identical and from the same factory. The outbreak was halted by shutting down the factory.

Dr. Peter Gerner-Smidt from the CDC has said that the preferred method PFGE is in the process of being replaced by NGS for PulseNet, which is used globally for the surveillance of foodborne diseases. This has worked very well, but it will become even easier to share information with NGS. Like PFGE, it's universal, electronic, portable and definitive.

Q: What are the next steps in your MRSA research?
HW: We are analyzing various MRSA, using our NGS and SNP trees to understand its epidemiology. MRSA is brought into Denmark by Danish people traveling around the world, and now we can say whether those isolates are identical or whether MRSA acquired in the Near East is different from MRSA acquired in India. There's a lot of evolutionary history of microbial-resistant strains that we can track with NGS. It's quite interesting.

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