Fighting antibiotic ‘superbugs’—DNA sequencing helps in the battle

The bacterium *Staphylococcus aureus (S. aureus)* has many faces. Many of us happily live our whole lives with S. aureus present on our skin or in our noses and experience no problems at all. But if the bacteria get further into the body they can cause health problems. These range from mild skin infections causing redness and blisters to life-threatening infections of the heart and lungs. In 2012, *S. aureus* was associated with 292 deaths in the UK.

Methicillin-resistant *S. aureu*s (MRSA) is a type of *S. aureus* that causes problems because it is resistant to the antibiotics that are normally used to treat these infections. This makes it much more difficult to get rid of so it is often referred to as a “superbug”. MRSA infections spread quickly in contained spaces, especially in nursing homes and hospitals where people often have weaker immune systems, enabling the infection to thrive. In hospitals MRSA can more easily enter the bodies of patients, thanks to cuts, wounds, and medical procedures. It is then harder to treat because of its resistance to antibiotics. However, increased awareness of MRSA has enabled healthcare professionals to manage it more effectively.

The Wellcome Trust Sanger Institute took DNA samples of *S. aureus* from around the world and used DNA sequencing to examine its transmission. It was one of the first times a study like this had been done, and the results, which were published in the journal *Science* in 2010, demonstrated the potential use of DNA sequencing to help reduce transmission and contain outbreaks of MRSA.

Isolating bacterial culprits

When scientists sequence bacteria they tend to sequence ‘isolates’. A swab is used to take a sample from a patient carrying the bacteria of interest. The bacteria from the swab are then grown on a plate in the laboratory. The bacteria will grow in different clumps on the plate, known as colonies. One of the colonies of the bacteria is selected and this is the ‘isolate’ from which DNA can be extracted and sent off for sequencing. Selecting isolates helps to ensure that only one type of bacteria is sequenced.

Colonies of S. aureus bacteria growing on a plate. Each white dot is a single colony.
Image credit: Pablo Rojas via Wellcome Images

Identifying different types of bacteria

For a long time, scientists used a method called ‘typing’ to trace the source of outbreaks of bacterial infection. The aim of typing is to find out whether two or more bacterial strains are related to each other and originate from the same source population (are of the same type). One of the most common techniques for doing this is called Multilocus Sequence Typing, or MLST for short. MLST involves sequencing around eight genes[?] in the genome of the bacterium. The sequence of these genes is then used to define the type of bacteria that is present in the sample. If the gene sequences are identical in two or more bacterial strains it means that they are of the same type.

More sequence – more information

Although MLST has been incredibly useful, we now have whole genome sequencing. This means that we can determine the DNA sequence of the entire genome of an organism like *S. aureus* in one go, all 2,600 genes. Whole genome sequencing can therefore reveal a lot more about different isolates than MLST that only analyses eight genes. This increased level of detail enables scientists to see the similarities and differences between individual *S. aureus* isolates.

Scientists at the Wellcome Trust Sanger Institute studied the relationships between *S. aureus* isolates from around the world using whole genome sequencing. Previously these isolates could only be separated into 10 seemingly identical groups based on their MLST profiles. With whole genome sequencing, each individual isolate could be distinguished from the others based on their genetic differences. By analysing the differences and similarities between the isolate's genomes, the scientists could also see how and where the different isolates evolved.

The scientists found that MRSA had been transmitted from Europe to South America and then back into Europe via Portugal. When they looked more closely at the data, they found an outbreak of MRSA in a UK hospital and a single case in Denmark that appeared to be closely related to MRSA isolates found in Thailand. After further investigation, the scientists identified that the isolate found in Denmark was in fact from a Thai person who had recently travelled from Thailand. This demonstrated that sequencing could accurately identify the source of an individual infection and how whole genome sequencing had the potential to be used in a clinical setting. The research also suggested that by using whole genome sequencing, scientists could find the source of a hospital outbreak and therefore stop it at its source before it becomes widespread. The scientists therefore set out to demonstrate this clinical application by looking at an outbreak of MRSA at The Rosie Hospital, part of the Cambridge University Hospitals Trust in the UK.

The special care baby unit outbreak

The Rosie Hospital is home to a special care baby unit (SCBU), which cares for babies that have been born early or with a low birthweight, as well as babies that are recovering from a difficult delivery, infection or surgery. These babies are very vulnerable to infection, therefore swabs are taken on admission and then every two weeks to monitor if they come into contact with any bacteria that could cause infections.

A special care baby unit. Image credit: N. Durrell McKenna via Wellcome Images

In 2011, three babies on the SCBU at the Rosie Hospital tested positive for MRSA. Although none of these babies were unwell as a result of the presence of MRSA, this prompted an investigation by infection control. The MRSA from each baby was tested against different antibiotics to determine their antibiotic resistance profiles. This type of test is called an 'antibiogram'. If samples of MRSA are resistant to the same antibiotics then they have the same antibiotic resistance profiles and are therefore more likely to be the same strain of bacteria. In this case, two of the MRSA samples had the same resistance profile and one of them differed by resistance to one antibiotic. The hospital then decided that the bacteria were

probably linked so carried out a deep-clean of the ward and investigated all MRSA-positive swabs from the previous six months.

During this period a total of 14 cases of MRSA were identified. Nine of the cases had the same, or similar, resistance profiles to the original outbreak, and five cases had different profiles and were considered unrelated.

The 12 related MRSA cases appeared in three clusters separated by 17 days and 33 days. Normally with transmission on a hospital ward, bacterial infection is passed directly from one person to the next with no noticeable gap, so these gaps of several weeks made it difficult to know if this was a single outbreak or several separate outbreaks.

At this point, scientists from the Wellcome Trust Sanger Institute used DNA sequencing to explore the outbreak in more detail. By comparing the genomes of each of the isolates they found that two of the five cases considered unrelated on the basis of their antibiograms alone were in fact related. They were also able to confirm that the three MRSA clusters were not separate but linked together and a single, ongoing outbreak. The question was why were there gaps of many days between them? Was the outbreak being repeatedly brought into the hospital from outside or was it originating from another ward in the hospital?

An illustration showing a timeline of the 14 genetically-related MRSA cases in the SCBU. (Data source: Harris et al. 2013; doi: 10.1016/S1473-3099(12)70268-2). Image credit: Genome Research Limited

Hunting for the source of the outbreak

To find out where the cases of MRSA originated, the scientists gathered MRSA samples from other wards in the hospital, as well as GP practices and clinics in the Cambridge area where patients had presented with symptoms of MRSA infection. They then performed antibiograms on the samples to find the ones that had similar antibiotic resistance profiles to the ones identified in the SCBU. Those samples with the same antibiogram then had their DNA sequenced.

When the DNA sequences of these isolates were studied a number of them were found to be closely related to the cases of MRSA on the SCBU. Two matching MRSA samples from GP practices were from babies who had been on the SCBU ward at the same time as some of the other babies, but hadn’t tested positive while they were on the ward. Not every swab will pick up infection each time so cases can sometimes be missed. There were also some women who went to their GP with abscesses (a symptom of Staphylococcal infection) who were the mothers of babies who were on the SCBU. One man in the community turned out to be the partner of one of these women.

The study found that there was no evidence that the outbreak had come from the community or other wards because all cases could be linked back to the SCBU.

Another case of MRSA was identified on the SCBU nine weeks later. It was presumed that this was a completely new outbreak of MRSA but when the DNA was analysed it showed it to be closely related to the previous cases of MRSA on the SCBU. This suggested that the MRSA was probably being transmitted by someone working on the SCBU but they would need evidence to confirm this.

An illustration showing a timeline of the 15 genetically related MRSA cases in the SCBU. (Data source: Harris et al. 2013; doi: 10.1016/S1473-3099(12)70268-2). Image credit: Genome Research Limited

Nipping it in the bud

Over 100 people who had worked on the SCBU were asked to provide a bacterial swab for DNA analysis. It is common for *S. aureus* and MRSA to be carried by people without any ill-effects so healthcare workers may sometimes unwittingly carry the bacteria. Out of all of the samples taken from SCBU staff, one tested positive for MRSA. When DNA from that isolate was sequenced it confirmed a link to the MRSA cases in the SCBU, suggesting the healthcare worker was the source of the most recent case on the ward.

Everyone on the ward received a series of medicated body washes, including the healthcare worker, to remove the MRSA. After three negative screens the healthcare worker was free from MRSA and could return to work. Finally, the outbreak was contained and eliminated.

But where did the healthcare worker pick up the MRSA? The most likely scenario is that one of the babies or one of the families was the original source of the MRSA infection and the healthcare worker was simply a carrier passing it between babies and families on the SCBU.

An illustration showing a timeline of the 15 genetically related MRSA cases in the SCBU and the healthcare worker that carried the infection. (Data source: Harris et al. 2013; doi: 10.1016/S1473-3099(12)70268-2). Image credit: Genome Research Limited

The MRSA clone involved in this case was investigated further and was found to be ST22, a strain of *Staphylococcus aureus* commonly found in UK hospitals. There are two main types of MRSA ST22 – one associated with hospitals, because it is resistant to particular antibiotics, and one associated with communities and not restricted to hospital environments. When the scientists looked in more detail at the isolates of ST22 found in the SCBU they were found to be more closely related to the community-associated type and similar to some found in South Asia, particularly India. It is only through DNA sequencing that they were able to find this out!

What next?

This was a landmark study, demonstrating how DNA sequencing could be used in a hospital setting, and showing how sequencing of bacterial genomes can be carried out on a large scale.

Initially this study was designed to show how DNA sequencing could provide information about a historical outbreak of MRSA in a hospital. However, DNA sequencing assisted in identifying that the outbreak was

ongoing and enabled the rapid identification of its origins. This enabled the efficient management and containment of the outbreak. This shows how DNA sequencing can help clinicians to prevent the spread of MRSA and limit the number of serious infections. In serious cases, some *S. aureus* infections can be fatal, others can require surgery to remove abscesses, but DNA sequencing could provide the insight to reduce the overall clinical burden of infectious diseases such as *S. aureus*.

As a consequence of this study and other research over many years, Cambridge University Hospitals Trust now routinely sequence the genomes of every case of *S. aureus* onsite to help keep one step ahead of any potential outbreaks. This could enable scientists to identify the genetic changes that cause resistance to antibiotics and use this information to inform doctors about which antibiotics to prescribe to their patients. This technique isn't limited to *S. aureus* and is also being used to tackle many other pathogens including tuberculosis and gonorrhoea.

Antibiotic resistance is frequently featured in the news as a growing global problem. Gonorrhoea for example is becoming almost untreatable due to antibiotic resistance. Gaining a better understanding of these bacteria with genomics and DNA sequencing is providing invaluable information to help advance research at a faster rate.

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