Case Study

Superbugs

Selecting our enemies

Version 1.1

Bronwyn Terrill, Steve Cross and Matt Holden
Wellcome Trust Sanger Institute
Hinxton
Superbugs
Selecting our enemies

Studies of the genomes of strains of *Staphylococcus aureus* have provided evidence for the rapid evolution of drug resistance. *S. aureus* is now best known to the UK public as its methicillin-resistant form, MRSA. Researchers have found that this bacterial species has evolved a ‘Pick’n’Mix’ genome that enables it to acquire new characteristics. The ability to share and exchange genes is vital to the *S. aureus*’s spread and success, presenting a major and constantly-changing clinical challenge.

In this activity, you will compare the genomes of an MRSA strain and its genetic ‘cousin’, MSSA, to locate DNA differences and mobile elements that could be expected to improve the bacterium’s resistance and its ability to cause disease.

**Overview of the activity**

Newspapers often carry frightening stories about ‘superbugs’ – ordinary bacteria that have developed resistance to many antibiotics. DNA sequencing technologies and genome analysis tools are now common ways of trying to understand the ‘arsenal’ of the disease-causing bacterium or pathogen.

In this activity, you will compare the genomes of two strains of *Staphylococcus aureus*, one of the UK’s best-known bacterial ‘superbugs’.
A complex bacterium

*S. aureus* is part of the natural flora of the human skin, carried by more than a third of the healthy UK population at any one time. It is a small, spherical bacterium and it is usually found on the skin or in the nose. Most of the time it doesn’t cause any illness in the people who carry it, but under some circumstances it can be responsible for a wide range of diseases: from pimples and food poisoning to toxic shock syndrome, heart infections and necrotising (flesh-eating) pneumonia.

Diseases caused by *S. aureus* are usually treated with antibiotics, and the patient is normally clear of the infection within a month.

How does it infect?

As you might expect from its ability to cause such varied diseases, *S. aureus* is capable of infecting a range of human cell types using different mechanisms. It has assorted proteins on its surface that attach to various human cell types, as well as some which help it clump together to attach to cells. It also releases toxins that damage cells and affect the immune system, making it harder for the body to deal with infections successfully. The final weapon in its arsenal are enzymes which allow the bacteria to weaken cell membranes and digest the cells’ contents.

The arsenal of weapons MRSA can use to attack its host.

**TOXINS**
- Hemolysins
- Leukocidins
- Enterotoxins
- Exfoliative toxins
- Toxic shock syndrome toxin

**ENZYMES**
- Lipases
- Esterases
- Nucleases
- Coagulases
- Phospholipase C

**SURFACE PROTEINS**
- Fibronectin binding protein
- Collagen binding protein
- Clumping factor
- Protein A
- Elastin binding protein
- Matrix adhesion factor

**EXTRACELLULAR PROTEINS**
**From infection to ‘superbug’**

Over the last decade, *S. aureus* has become more than a relatively harmless bacterium that many of us carry around. Antibiotic-resistant strains have been associated with significant increases in healthcare-associated infections in UK hospitals. Multiple antibiotic-resistant strains of bacteria such as *Staphylococcus aureus* and *Clostridium difficile* (Cdiff) are now popularly known as ‘superbugs’. *S. aureus* is starting to kill people in greater numbers, because of the increased virulence of new strains, and the fact that many of them can resist antibiotics.

**Antibiotic resistance**

The use of antibiotics, starting with penicillin in the 1940s, has created a new selection pressure on bacteria. Researchers believe that many bacteria have evolved increased resistance to various types of antibiotics as a result. Where an antibiotic is used to treat an infection, any bacteria present that are resistant, because they have mutated or acquired new genes, will eventually come to make up the whole population. The first antibiotic-resistant bacteria were isolated in 1947 — just four years after penicillin went into mass-production.

Bacteria often exchange genes, either through the transfer of plasmids (rings of DNA which sit outside the main chromosome) or via phage (viruses that can infect bacteria and lie dormant in their chromosomes). This ‘horizontal’ gene transfer (in other words, not just down the generations, but within them) allows resistance to spread even faster than it would through a mechanism of simple mutation and selection.

**Making sick people sicker**

MRSA is now considered endemic — a regular presence — in UK hospitals. In 2006, about 17,000 people admitted to hospital in the UK acquired *S. aureus* infections, of which about 7,000 were MRSA. This places extra pressure on the health service, because infected people had longer hospital stays at an estimated additional cost of £1 billion per year.

The antibiotic ‘of last resort’, used to treat patients whom methicillin cannot help, is vancomycin. Vancomycin-resistant bacteria are also beginning to be isolated in hospitals.

In the UK, the Health Protection Agency is responsible for monitoring all hospital-acquired infections. You can read more about infection rates and attempts to control MRSA in UK hospitals on the HPA website: [www.hpa.org.uk](http://www.hpa.org.uk)
Community-acquired MRSA

On rare occasions, people are infected with MRSA without going into hospital. These infections are known as community-acquired MRSA. In the UK, there are 30–40 cases a year. In other countries, especially the USA, such infection is far more common, usually occurring in places where people spend a lot of time in close contact, such as massage parlours, or where they engage in activities which regularly graze and break the skin, such as contact sports. These grazes provide a route for MRSA to enter the body and cause infection.

Some community-acquired strains of MRSA are especially virulent. These include the Panton-Valentine Leukocidin (PVL)-positive strains of MRSA that are sometimes reported in the news. PVL is a toxin produced by bacteria that attacks white blood cells and it can cause serious abscesses, boils and tissue necrosis (death of body tissues). In extreme cases it can cause septic arthritis, septicaemia or the deadly necrotising pneumonia.

Fortunately, community-acquired MRSA infections tend to be less resistant to antibiotics other than methicillin than hospital-acquired infections, so they can often be treated relatively easily.

Studying S. aureus genomes

Bacteria are far more genetically-diverse than we ever expected and show much more variation than human beings. In two strains of a single species, up to 1,000 genes can differ. We, on the other hand, only have a few genes that are different from those of a mouse. Because bacterial genomes change so rapidly, they evolve equally rapidly and we can study that by looking at the detail of their genomes.

Scientists who use genomics to find out more about bacteria often take several different approaches to uncover useful information to prevent disease:

1. By completing the gene map of a bacterium, it is possible to locate within a pathogen's genome all the genetic ‘weapons’ that they are using to attack host cells. This can provide information about how disease is caused by the bacterium, and so help doctors understand which treatments would alleviate symptoms. On a more fundamental level, some of the genes or proteins identified in a full genetic map may become targets for new drugs or for vaccines, to help prevent or clear up infections.

2. By comparing strains of bacteria to study their evolution. Antibiotic-resistant strains have often acquired genes that their antibiotic-sensitive ancestors didn’t have; they may also have lost genes in the process. By studying the genomes of these two groups and comparing them, scientists can start to understand how strains develop new characteristics.
The genomes of *S. aureus*

The *S. aureus* genome is approximately 2,800,000 base-pairs in length. More entire sequenced genomes are now available for different strains of *S. aureus* than for any other bacterial species. These include strains obtained from infected patients all over the world. Each of these sequences has a large set of conserved genes, alongside a number of mobile genetic elements. These elements contain the individual toxin, cell-surface protein and resistance genes that give each strain its infectiousness.

Mobile genetic elements

Bacteria can gain genes in several ways — often through carrying or incorporating different types of mobile genetic elements. Mobile genetic elements are pieces of DNA which are capable of moving around within a genome, being duplicated within a genome at remote sites or of being transferred to another bacterium’s genome. They take a variety of forms, usually carrying the instructions to make some of the proteins needed for their own transfer.

Some of these elements are really bacteriophage (viruses that attack bacteria) lying dormant in the genome, waiting to move or to be converted back to a viral form. When a mobile genetic element contains several related genes such as those conferring antibiotic resistance, it is known as a cassette. Such cassettes are often transferred between bacteria.

As well as these cassettes and mobile elements within their genome, many bacteria also carry plasmids, which are rings of DNA that exist outside their main chromosome. Plasmids often carry non-essential genes like those that confer antibiotic resistance and can, in many cases, be copied and transferred between bacteria, even those of different species.

Plasmid DNA conferring antibiotic resistance can be transferred between bacteria by a natural process called conjugation.

**STEP 1:** Plasmid DNA conferring antibiotic resistance is unwound and replicated.

**STEP 2:** Single-stranded DNA passes into the recipient cell through the pilus.

**STEP 3:** Complementary DNA is synthesised, forming a new plasmid.

Sometimes the resistance gene is incorporated into the recipient cell’s chromosome.
**Exercise 1**

**Computer-based genome comparison of antibiotic-resistant and sensitive strains of *Staphylococcus aureus***

In this activity you will examine the genomes of two very closely-related strains of *S. aureus* to explore how a methicillin-sensitive strain has developed resistance to the antibiotic.

You will:
1. compare the sequences of the two genomes to look for large regions that have been inserted or deleted;
2. examine some of the genes within those differing regions to find out what they do.

Both genome sequences have been provided for you to view. They have been compared using a piece of software called ‘ACT’ which stands for the *Artemis Comparison Tool*.

The two strains to compare are:

1. **MSSA476** — a methicillin-sensitive *S. aureus* strain, isolated in 1998 from a 9-year old boy with a community-acquired infection. This strain is resistant to the antibiotics penicillin and fusidic acid, but can be killed by many other antibiotics. After antibiotic treatment, the patient made a full recovery. The 2,799,802 bp genome of MSSA476 was fully sequenced in 2004 by staff at the Wellcome Trust Sanger Institute.

2. **MW2** — This strain of *S. aureus* is resistant to methicillin. It was isolated in 1998 from a 16-month-old girl with severe infections. MW2 is sensitive to some types of antibiotic, but it is so effective at causing disease (so virulent) that the baby girl died within two hours of arriving in hospital. The genome of MW2 was fully sequenced in 2002 by staff of Juntendo University in Japan.

**Looking at the whole genomes**

Using the *Artemis Comparison Tool (ACT)*

ACT is a piece of software, developed by the Wellcome Trust Sanger Institute, for visualising the results of a computer-based comparison of two DNA sequences. In this case, the two sequences are compared automatically using software called *BLAST*, which attempts to line up the sequences where they match.

Rather than give you the entire file to flick through (and get lost in), we’ve created a simplified, animated version in *Adobe Flash* with the information that researchers think is important.
In the ACT display, the different strains are represented by grey lines across the screen (their names are on the left). For example, in the screenshot above, the methicillin-sensitive strain, MSSA 476, genome is the first from the bottom. Above it is the methicillin-resistant strain, MRSA MW2.

Where the two genomes are the same, ACT joins them with red lines. Where they have the same sequence, but one genome has the sequence reversed (an inversion), it joins them with blue line. A section of either genome that matches nothing in the other genome will look white.

Start the Flash version of the ACT tool. In this version, just two strains are shown:

The *methicillin-sensitive* strain, MSSA 476 genome is across the top.
The *methicillin-resistant* strain, MRSA MW2 genome is across the bottom.

A screenshot of the ACT, showing five different bacterial strains.

A screenshot of the Adobe Flash version of ACT, showing a comparison of two different bacterial strains.
Questions

a. From the overall view of the two genomes, how similar do you think they are?
b. How many mismatched areas (patches of white) can you see at this stage?
c. Are there areas in both MSSA and MRSA that don’t match?

In-depth analysis

Click on the overall comparison view to zoom in. You can scroll through the entire comparison using the bar at the base of the screen. Click Help (at the top right of the screen) for more information.

There are many regions in the MRSAMW2 and MSSA476 strains that differ: these might be inserted sequences, deleted (or missing) sequences, or the same sequences in different genomic positions. We have provided a clickable coloured box by each of the regions that have been inserted into the MW2 genome. If you click on a box, you’ll see a zoomed-in version of the comparison in that region.

Zoomed in regions

ACT displays genes in each genome along the top and bottom as coloured boxes. To zoom in on these areas, click the boxes. To simplify the image, we have chosen not to display genes outside the major regions of inserted or deleted sequence.

If you click on the name of a gene, you can bring up a box that tells you about it. Some of the genes have known functions, and some are predicted by the software to be genes. Any gene name that starts MW is hypothetical, so there isn’t any information on what it does.
Examining the genes
The software has been set up to provide you with DNA and amino acid sequence for each of the interesting genes in these regions. For many of the genes, you will see a link to the RCSB Protein Data Bank (www.pdb.org) that you can click or copy into a web browser to view a relevant protein structure. We recommend clicking on the word ‘Jmol’ under the image on the right of the screen to see a 3-D version of the structure without having to download extra viewing software.

Screenshots of the Adobe Flash version of ACT. Clicking on the gene names will bring up additional information and links.
The DNA and amino acid sequences can be cut-and-pasted from the results pages for use in, for example, BLAST searches to find other similar bacterial genes.

Explore the genome region by region, to work out which genes are found in the methicillin-sensitive and methicillin-resistant strands. Use this information to develop your ideas about the two different strains of S. aureus.

Questions

c. In the first clickable area (region 1) there are some small sections of DNA present in both strains, and larger sections which do not match. Can you think of different ways this might have happened?
d. Can you find any genes in this region that might contribute to either strain’s ability to cause disease?
e. In the second major region where the two strains differ, you can see there are several genes in the region of MW2 which do not match any part of MSSA476. Once again, try clicking on the gene names in MW2 to find out more. What do these genes do and how might they contribute to MW2’s resistance and ability to cause disease?
f. What do they tell you about this region of the genome and how it may have been acquired?
g. In the third and fourth clickable regions (regions 3a & 3b), the majority of the blue lines on the comparison of the two genome sequences represent a bacteriophage which is present in both genomes, in different places and in the opposite orientation. Although most of the sequence is identical in each genome, there is a region of difference. Examine the genes in this region. Are any of these genes likely to contribute to the bacterial strain’s disease-causing abilities (virulence)?
h. What do the genes suggest about how this region may have been acquired?
Exercise 2
Categorising the genes of antibiotic-resistant and sensitive strains
(an offline activity)

Look through the set of printed gene descriptions that your teacher will make available to find genes you looked at in the MSSA/MRSA comparison.

Separate the genes found in the MSSA and MRSA strains, then separate the genes into functional types e.g., antibiotic resistance, toxins, cell surface proteins, mobile genetic elements (transfer genes) etc.

Questions

i. Which of these genes would make a bacterium better at causing disease?

j. Which of these genes might give a bacterial strain some kind of advantage over other strains?

k. Based on these two very similar genomes, which mechanisms do you think are driving the evolution and spread of antibiotic-resistant bacteria?