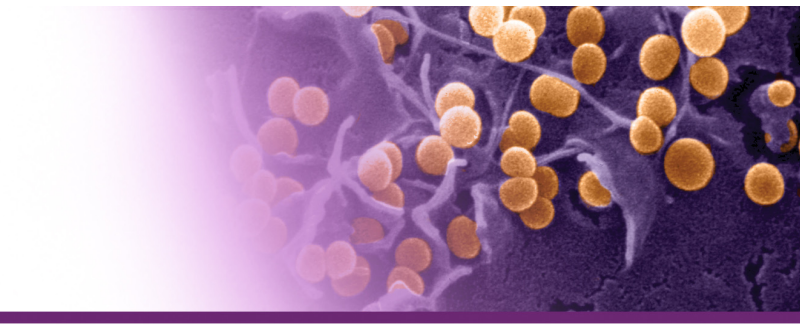
MRSA gene hunt
(Gene ID card version)
Teachers' notes



Background to the activity
This a classroom based activity that allows students to explore features of two bacterial pathogen genomes. The aim of this activity is to highlight the role of different genes in the virulence, pathogenicity and evolution of *Staphylococcus aureus* bacteria strains. Using gene identification cards students research and classify key genes that play an important role in drug resistance and virulence for two strains of *S. aureus* (MSSA 476 and MRSA MW2).

Estimated duration: 60 minutes including presentation and discussion

To run the activity you will require:
- Student worksheets with introductory information
- Introductory PowerPoint
- Gene ID cards
- Flipchart paper
- White board
- Reusable adhesive e.g. Blue Tack®
- MRSA animation (optional)

Classroom activity preparation
The following components need to be prepared before the activity commences:

1. Gene ID cards
Print off all of the gene ID cards. One set of cards is needed per group of three to four students. Cut out, fold and stick the two sides together so they become a double sided card, with information on one side and gene name on the other. It is recommended that the cards are laminated to prevent damage, however this is not essential.

2. Summary tables
In order for the students to summarise their classification, you can choose to prepare A1 summary charts in advance or you can ask the students to do this as part of the activity. Using one sheet of flipchart paper (landscape orientation) draw a table with the headings shown below:

Antibiotic resistance	Enzyme	Surface protein	Toxin	Mobile genetic element

This will be used by the students to group the gene cards and feed back to the rest of the class.

PowerPoint presentation
The presentation introduces the activity, some essential terminology and background information on *S. aureus* biology and the mechanisms by which it can cause disease. This provides the students with the information they require to complete the activity. The presentation also includes the results from the activity and questions to encourage further consideration and discussion of the data.

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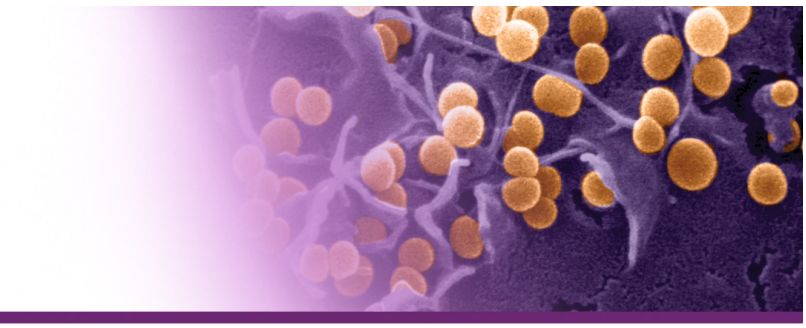


Instructions for running the activity

About the Gene ID cards
The gene ID cards contain details of genes from two strains of *S. aureus*:

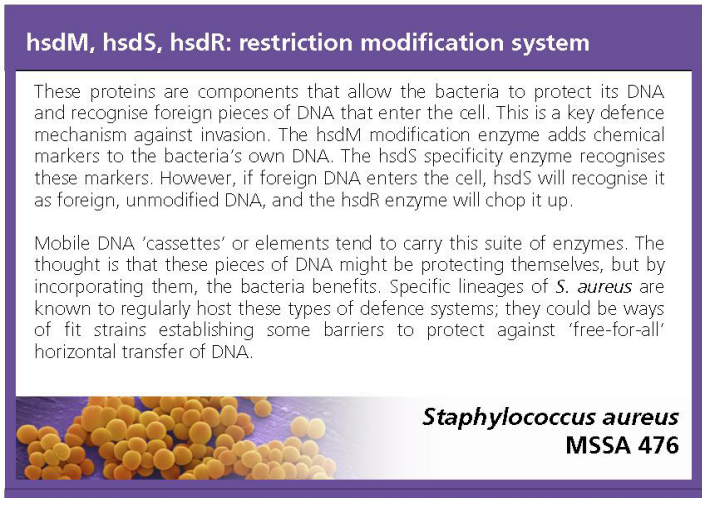
MSSA 476
This is a methicillin-susceptible strain of *S. aureus* that was isolated from a 9-year old boy with a community-acquired infection in 1998. This strain is resistant to penicillin and fusidic acid, but can be killed by many other antibiotics. After antibiotic treatment, this patient made a full recovery. The 2,799,802 bp genome of MSSA 476 was fully sequenced by staff at the Wellcome Trust Sanger Institute in 2004.

MRSA MW2
This strain of *S. aureus* is resistant to methicillin. It was isolated in 1998 from a 16-month old girl with severe septicaemia (blood poisoning). MW2 is sensitive to some types of antibiotic, but is extremely virulent; this patient died within two hours of arriving in hospital. The genome of MW2 was fully sequenced in 2002 by staff at Juntendo University in Japan.

Group size
The task can be carried out in groups of up to four students.

Each group will require:
- one set of gene ID cards
- four worksheets (one per student)
- one sheet of flipchart paper with summary chart (or blank paper if you want the students to create their own summary chart)
- reusable adhesive e.g. Blue Tack®

Interpreting the Gene ID cards



Gene ID: gene name

Description of the protein product and function

Strain of bacteria

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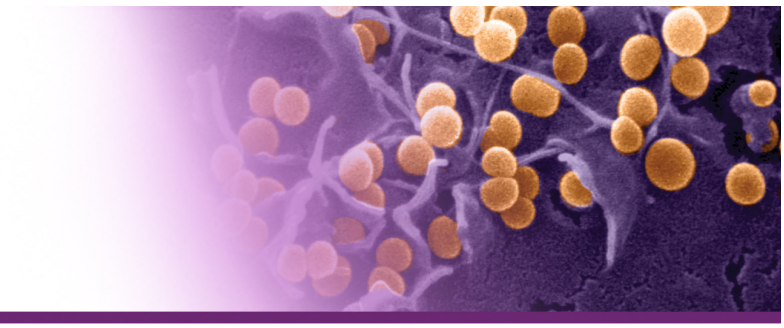

Completing the worksheet

The worksheet has a list of 14 different genes which can be found in the genome of the two strains of *S. aureus*. The students should use the gene ID cards to describe their roles and to classify the protein product they code for.

All of the information can be found on the gene ID cards. The table can be completed as shown below:

Gene	Protein product	Role /Function	MSSA 476	MRSA MW2	Classification	
ccrA & ccrB	Cassette chromosome recombinase A	Allows a genomic "cassette" to be inserted into the bacterial chromosome. This helps the bacteria to acquire new genes	✓	✓	Antibiotic resistance Enzyme Surface protein Toxin Mobile Genetic Element	□ □ □ □ ✓

- The gene name is the label at the top and back of the gene ID card.
- The protein product can be interpreted from the information provided on the gene ID cards as can the role of the protein.
- The presence of a gene in each bacterial strain is indicated by the labels at the bottom of the gene ID card. Where genes are present in just one genome, only the relevant strain should be ticked.
- Using the information provided, students should classify the gene and protein product into one of five categories by ticking the relevant box.

Summarising the findings

Each group should be provided with a sheet of A1 flipchart paper so that they can categorise the genes. This can be prepared in advance (see page 1, Classroom activity preparation) or you can instruct the students to create their own summary table using the categories on the worksheet.

Students should stick the gene cards (label side up) under the gene categories with reusable adhesive to summarise their findings. These can be used to feed back to class discussions.

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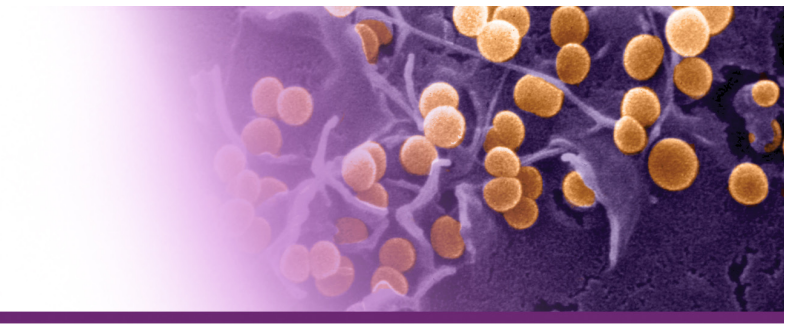


Answers and discussion points

The answers are provided in grid form in the gene classification answers document, they are also on the presentation provided. Start the discussion with the first question from the activity sheet:

Q1a) Which genes are found in MSSA 476?
Eight genes can be found in the MSSA 476 genome. These are: *ccrA & ccrB, int, fusB1, hsdR, hsdS & hsdM* and *orfX.*

Q1b) Which genes are found in MRSA MW2?
Nine genes can be found in MRSA MW2 genome. These are: *ccrA & ccrB, ear, orfX, lukS, lukF, mecA, sec4* and *sel2.*

Q1c) Which genes are found in both strains?
Only three of the genes are found in both strains. These are *ccrA & ccrB* and *orfX*

Use slide 27 of the presentation to explore how the students have classified the genes. The slide is animated to allow step-wise revealing of the answers.

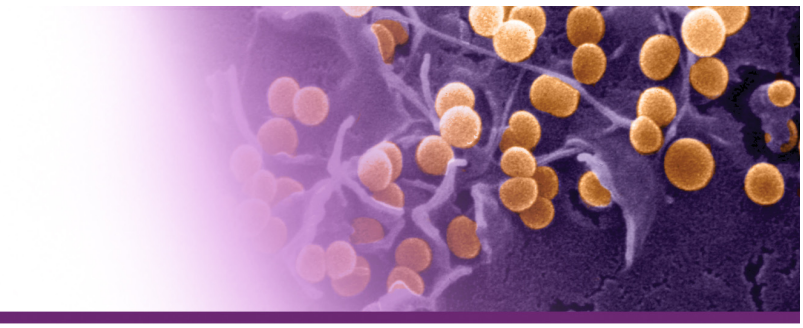
Antibiotic resistance	Enzyme	Surface Protein	Toxin	Mobile genetic element
•*fusB1* •*mecA*	•*hsdR, hsdS & hsdM*	•*orfX*	•*ear* •*lukS* •*lukF* •*sec4* •*sel2*	•*ccrA & ccrB* •*int*

Q2. Which genes could be termed as virulence factors, i.e. factors that give the bacterium a greater capacity to cause disease?
The enterotoxin genes *ear, sec4 and sel2* and cytotoxin genes such as *lukS* and *lukF* are key virulence factors. These toxins have the capacity to destroy critical cellular components such as the cell membranes and kill cells such as leukocytes (white blood cells).

Panton–Valentine leukocidin (PVL) is a cytotoxin, that works by forming holes in the cell membranes of leukocytes (white blood cells) causing their cell contents to leak out and cell death. It has recently been associated with community-acquired MRSA infections (infections which originate outside of the hospital environment) and necrotising pneumonia.

PVL consists of two protein subunits called lukF and lukS which combine to form a pore or hole in the membrane of the leukocyte. By attacking and stimulating these immune cells, PVL causes a strong immune response that leads to extensive damage to healthy tissues.

Necrotising pneumonia is a serious and often fatal disease which affects young patients with compromised immune systems. It is characterised by a decrease in the levels of white blood cells (leukopenia), which are killed by PVL, coughing up blood (haemoptysis) and the extensive build up of necrotic or dead tissue in the lungs due to damage by the over-stimulated immune system.



Some enterotoxins for example **enterotoxin B** (*ear*), can act as powerful superantigens if released in other parts of the body. Superantigens stimulate the immune system to over-produce T-cells (a type of lymphocyte which recognises and destroys pathogen antigens). If released in the respiratory tract enterotoxin B can cause a range of respiratory problems from chest pain to pulmonary oedema (fluid in the lungs), which can lead to failure of the respiratory system.

Q3. Which genes might give one strain an advantage over other strains?
Antibiotic resistance genes can give bacterial strains a selective advantage over other non-resistant strains because they cannot be killed by conventional antibiotics. This means that alternative therapeutic strategies are needed to treat antibiotic resistant bacterial infections.

Fusidic acid is a common antibiotic that targets elongation-factor G (EFG). EFG has a key role in protein synthesis and is encoded for by the gene *fusA*. Resistance to fusidic acid can arise from point mutations in *fusA*, or the bacteria acquiring the *fusB1* gene. The *fusB1* gene codes for a protein which binds to and protects EFG. Community-acquired MSSA 476 has this gene. This gives the strain a selective advantage outside of hospitals which presents therapeutic challenges when fusidic acid is prescribed.

Mutation fact
"Single nucleotide polymorphisms (SNPs) are more likely to have functional effects if they occur in the first or second base pair of the codon. Redundancy in the amino acid coding system means that the nucleotide in the third base position of a codon can be changed without affecting the amino acid it encodes" Lindsay & Holden, 2006**.**

Q4. What mechanisms do you think underlie the evolution and spread of antibiotic resistance in bacteria?
The use of antibiotics has created a new selective pressure on bacteria. Where an antibiotic is used to treat an infection, any bacteria present that are resistant, will eventually come to make up the majority of the population. The first antibiotic-resistant bacteria were isolated in 1947 – just four years after penicillin went into mass-production.

How do bacteria acquire antibiotic resistance?
Antibiotic resistance can occur through a process of point mutations, a series of base pair changes which can confer antibiotic resistance to the bacteria. For example fusidic acid resistance in some *S. aureus* clinical isolates has been attributed to mutations in the *fusA* gene (mentioned above).

Bacteria often exchange genes, either through transfer of plasmids (little loops of DNA which sit outside of the main chromosome) or via phage (viruses that can infect bacteria and lie dormant in their chromosomes). *fusB1* is an example of this.

This horizontal gene transfer allows resistance to spread rapidly through the population of bacteria. The mechanism of horizontal transfer spreads antibiotic resistance through a population much faster than point mutations which arise in one bacterium only and spread by division of that cell. Slide 17 of the presentation illustrates this.

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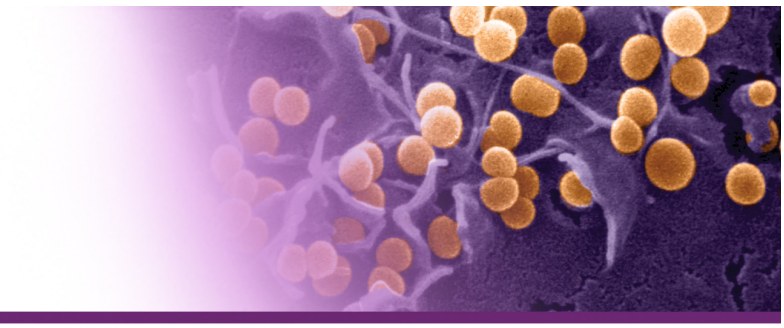

Discussion point

MRSA can be treated with the antibiotic vancomycin. However, in 2003 the first vancomycin resistant strains of MRSA (VRSA) were discovered. What are the implications of this?

If vancomycin is ineffective at treating the MRSA infection other treatment strategies are considered. Current treatment strategies for vancomycin resistant infections focus on surgical techniques to remove the bacteria and infected tissues to stop the infection spreading further into the body. These surgical techniques may include:

- surgical drainage where a tube is placed in the wound to remove pus, blood or other fluids
- debridement where tissue that is infected or dead is cut out and removed
- amputation.

Encourage the students to consider what the implications of surgical removal are for the patient and the NHS? You may discuss the following points:

- Extended stay in hospital, increased cost of treatment, risk of secondary infections, increased risk to the patients through the use of other drugs, anaesthetics etc.
- Also consider the psychological and functional impacts of such procedures, e.g. scarring and amputation.

Long term strategies for the treatment of vancomycin resistant MRSA focus on research into the development of new drugs or new drug combinations that target both MRSA and VRSA. Encourage the students to consider and discuss the following points:

- Can new drugs be developed for VRSA? If so will the bacteria develop resistance to these new drugs? How do you prevent bacteria from developing resistance?
- Could you develop vaccines to remove *S. aureus* completely from humans? What could be the implications of this?
- How does 30% of the population co-exist with *S. aureus* without becoming ill? Would removing *S. aureus* from humans open the way for other pathogens?

References

- Besier et al. Molecular analysis of fusidic acid resistance in *Staphlycoccus aureus*. *Molecular Microbiology*, 2003; 47: 463-469.
- Lindsey & Holden. Understanding the rise of the superbug: investigation of the evolution and genomic variation of Staphylococcus aureus. *Funct Integr Genomics*, 2006; 6: 186-201.
- Hiramatsu. Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance. *Lancet infectious disease*, 2001; 1: 147-155. (http://www.antimicrobe.org/Lancet5.pdf).