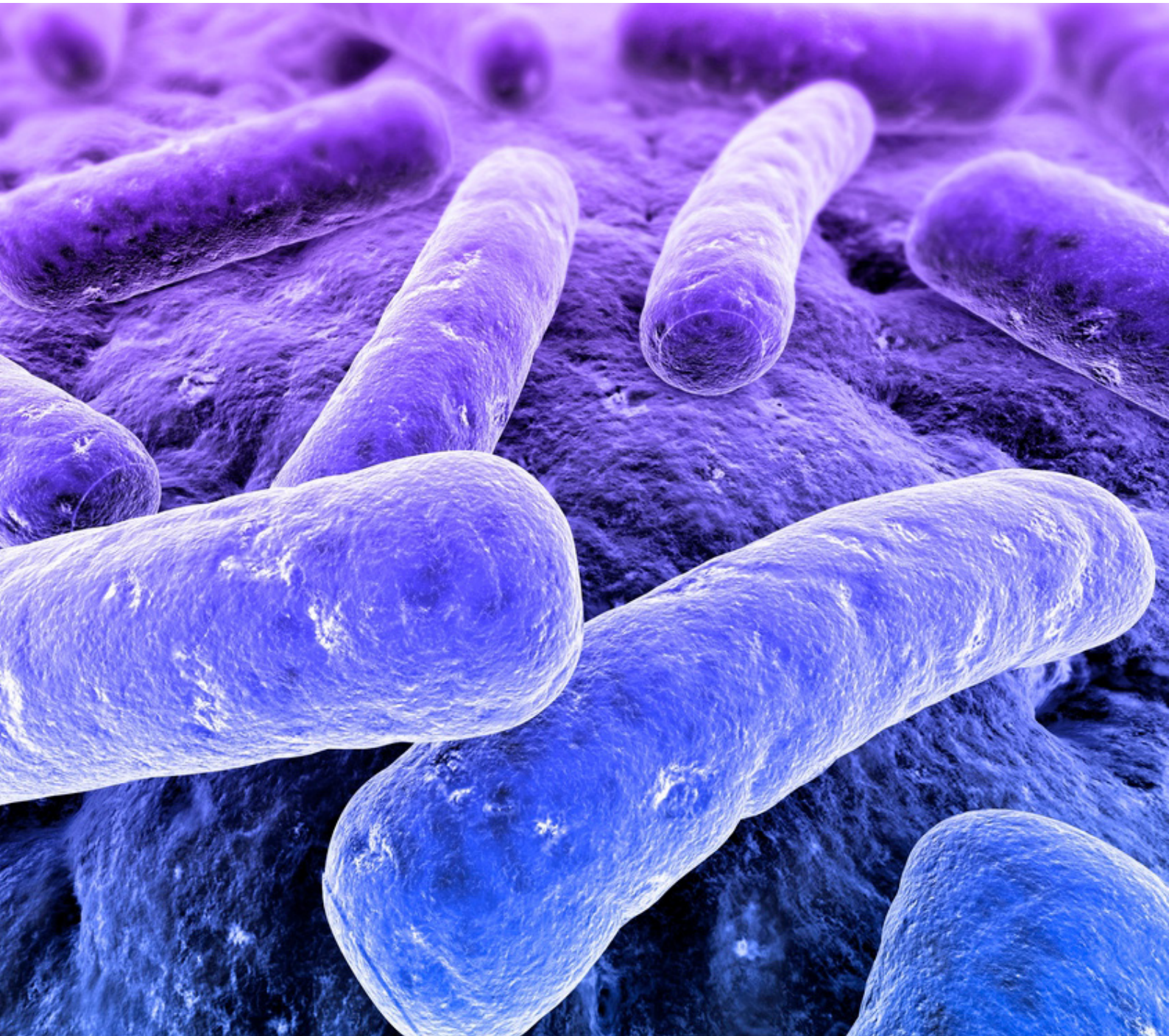
BIOCHEMICAL
SOLUTIONS TO...

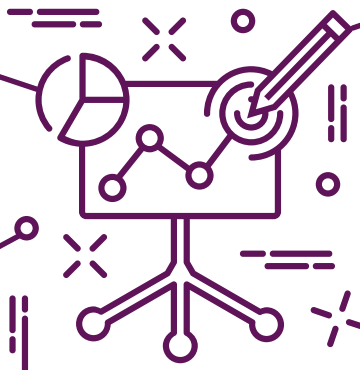
ANTIBIOTIC RESISTANCE



DESIGNING YOUR GOLD CREST PROJECT

This pack includes background information and examples of antibiotic resistance and the use of biochemistry in identifying solutions. It also provides examples of lab-based practicals and computer-based research projects to help contribute to designing your own Gold CREST Award project.

To achieve your Gold CREST Award you are required to meet a set of criteria. This resource sheet is designed to work towards achieving your award.



You are expected to design, plan and carry out an investigation with a clear set of aims involving a **minimum of 70 hours of work**.

This project should be self-directed and contribute something unique to the scientific community. You are expected to organise your time effectively and demonstrate a high level of understanding of the topic and a clear strategy to solve the problem you are addressing.

Your final report will include background information, progress of your investigation, results/findings, conclusions and reflection. You need to present your findings in an understandable manner using an appropriate medium.

Look at the **CREST Awards site** for more information about the required criteria.

DESIGNING YOUR GOLD CREST PROJECT

If you plan to design and implement a lab-based experiment, have a look at our examples here: Project example **1 'Looking at natural antibiotics'** and example **4 'Looking at prebiotics'**.

If you are wanting to design an investigative research project, have a look at project example **3 'Looking at probiotics'** and project example **5 'Preventing an outbreak'**.

If you want to incorporate bioinformatics into your project, look at project **example 2** as a starting point to your investigation, giving you some ideas of websites you can use.

You can use any of these examples as starting points for your own projects or just as ideas of other projects.

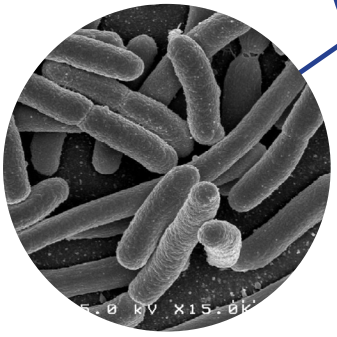
You can combine any of these examples to create a project that involves different areas of antibiotic resistance using physical experiments as well as web-based research projects to make your own unique project.

BIOCHEMICAL SOLUTIONS TO...

ANTIBIOTIC RESISTANCE

WHAT ARE BACTERIA?

Bacteria are single cell organisms that are spherical, spiral or rod-shaped and appear individually or in chains/clumps. Bacteria (prokaryotic cells) are found everywhere and are surrounded by a cell membrane and a cell wall but lack the internal organelles and nucleus found in eukaryotic cells, such as human and plant cells. Bacteria can be pathogenic and cause disease (for example tuberculosis), or can be non-pathogenic and not cause disease (for example *Lactobacillus* spp. found in milk). Non-pathogenic bacteria can be beneficial for human health.



WHAT ARE ANTIBIOTICS?

Antibiotics are drugs used to treat or prevent bacterial infections. They work by killing or inhibiting the growth of bacteria. Penicillin, the first antibiotic, isolated in 1928 by Alexander Fleming is used to treat streptococcal and staphylococcal bacterial infections. Modern medical techniques including surgery, transplantation, preterm babies and chemotherapy would not be possible without antibiotics. What other examples can you think of? A lot of antibiotic treatment today includes the combination of multiple antibiotics to target a bacterial infection, this is because antibiotics don't tend to be specific enough to kill a bacteria independently. There are different types of antibiotics that work via different mechanisms - they tend to be bactericidal (killing the bacteria) or bacteriostatic (slowing the reproduction of the bacteria). The more antibiotics are used, the more quickly the bacteria develop resistance. Bacteria are becoming increasingly resistant to these drugs creating large problems in medicine and agriculture.

WHAT IS RESISTANCE?

Bacterial resistance refers to when the antibiotic is no longer effective against the bacteria it is trying to kill. Resistance is when bacteria fundamentally changes physically, resulting in the reduction of the effectiveness of the drugs. Resistance arises when changes (mutations) occur in the DNA sequence of the bacteria that give the bacteria a survival advantage. These changes occur randomly within the genome. If a mutation occurs within a gene important in the bacteria's structure for example, this may result in the bacteria changing and evading the antibiotics; this is now a beneficial resistance allele.

This results in the bacteria surviving and continuing to grow, spreading this advantageous mutated resistance allele to other bacteria. Resistance can spread to the next generation by vertical evolution, passing the genes on to the next generation, or to other bacterium by horizontal gene transfer. Horizontal gene transfer can occur via the process of conjugation, where one bacteria (donor) transfers genetic material to a second bacteria (recipient) by direct contact of the cell walls.

We are entering a dawn of a post-antibiotic era. Our future relies on YOU to undertake a quest to find new treatments for drug resistant bacteria with the utmost urgency!

WHAT ARE THE CAUSES?

1. OVER-PRESCRIBING

Overuse and misuse of antibiotics for the wrong bacterial infection or when it is not required, leads to bacteria becoming resistant. The more we use antibiotics, the quicker the bacteria develop resistance! It can lead to the bacteria with antibiotic resistance alleles surviving when the antibiotic is applied - they have a selective advantage. This means that the number of bacteria with antibiotic resistance increases over time as the antibiotic is given.

FACT.

1 in 5 hospital emergency room visits are from medication errors with antibiotics.

2. NOT FINISHING TREATMENT

Underuse of antibiotics with patients not finishing the course of antibiotics results in the bacterial infection lingering and developing resistance to the antibiotic. Again, if the bacteria isn't completely removed, you increase the chance of the surviving bacteria having undergone random mutations that then survive and go onto to transfer the resistant alleles. This results in the effectiveness of the drugs being lost.

FACT.

8 million extra days were spent in hospitals due to resistant bacteria.

3. POOR HYGIENE AND SANITATION

To reduce the spread of resistant bacteria, good hygiene and sanitation are required – examples include body cleanliness, hand washing and clean clothing to reduce the likelihood of harbouring bacteria that can then be easily spread! Germs can spread easily by touching something someone with an infection has touched. Hygiene can be limited in countries with lack of clean water.

FACT.

Antibiotic resistant bacteria causes 23,000 deaths annually (2017).

WHAT ARE THE CAUSES?

4. SLOW DEVELOPMENT OF NEW ANTIBIOTICS

It can takes years to discover, develop, test and get approval for new drugs. As the resistance of bacteria is rapidly increasing, it can be hard for scientists to keep up! New resistant strains are constantly popping up making the drugs un-usable!

FACT.

Only 2 new classes of antibiotics introduced since 1968!

5. OVER-USE IN LIVESTOCK AND FARMING

Sub-therapeutic (low drug dosages, below the level necessary to treat the disease) doses of antibiotics are used in animal rearing to prevent diseases and promote growth in the animals. This increases the likelihood of the bacteria becoming resistant to antibiotics. This can result in resistance that spreads to humans.

FACT.

£29.9 million was spent on antibiotics in food production (2011).

6. SUB-OPTIMAL CONTROL OF HOSPITAL INFECTIONS

Hospital patients can be a main source of antibacterial resistant strains. This is due to illness and over-use of antibiotics. With over-populated hospitals and strain put on the hospitals to control infections, these patients become a source to spread these strains among the general public.

FACT.

The next pandemic will likely involve antibiotic resistant superbugs, one of those bacteria that cannot be killed using multi antibiotics, otherwise known as multidrug-resistant bacteria!

HOW CAN WE SOLVE THIS PROBLEM?

CASE STUDY: USE OF THE MICROBIOME

We live with bacteria in a mutualistic relationship where we (the host) provide a home for the bacteria, to survive, feed and reproduce, and the bacteria, in return, 'carries out beneficial reactions and processes. One example of this is in our immune system. Human immune systems and the microbiota (bacterial flora) engage in 'cross-talk' via chemical signals. This results in the immune system recognising the microbiota and only attacking harmful foreign bacteria. In return, the microbiota help the host by influencing immune reactivity and targeting. Maintaining a balanced and diverse microbiome is essential for staying healthy.

Finding new ways of tackling the problem of antibiotic resistance is essential. Over 16 million antibiotic prescriptions are made each year to NHS patients to treat infections in the lower lung alone; for example treating lung infections like Pneumonia. Lung infections are common in people with cystic fibrosis making it a primary driver of antibiotic resistance. People with cystic fibrosis tend to have a weakened immune system and compromised lungs, making them susceptible to lung infection and therefore rely heavily on antibiotic treatment. Increased antibiotic treatment increases the likelihood of their lung microbiome developing subsequent resistance.

Unfortunately, antibiotics aren't specific to one type of bacteria but target a range of bacteria. This means, when antibiotics are prescribed, they target more than the infecting bacteria. With high levels of bacterial infection, high numbers of prescriptions and a lack of antibiotic specificity, microbes in the lungs and gut adapt and become resistant. This changes the microbiome, potentially killing off some of the 'good' bacteria and allowing the survival of some of the 'bad' bacteria that are likely to obtain resistance and could lead to further and more dangerous infections. If we could change the gut microbiome of individuals to replace all the resistant bacteria with good healthy bacteria that aren't resistant, perhaps we wouldn't need to develop new drugs and we could continue to use existing ones!

A lot of research has been done to identify ways to guide the diverse microbiota which inhabits the human colon, particularly the bacteria required to allow fermentation of undigested food. The concept of 'prebiotics' was developed in the 1990s to help guide the microbiota to benefit the host. Prebiotics are compounds in food that induce growth or activity of beneficial bacteria. These 'prebiotic' supplements have been designed to tailor the human gut and usually include fibre. Natural examples include raw garlic and raw leeks. Research is still needed to understand the mechanisms and properties of probiotics.

You can learn lots about the microbiome and the uses of the knowledge. Check out **https://learn.genetics.utah.edu/content/microbiome/**.

EXAMPLE PROJECT IDEAS

A PRACTICAL BASED PROJECT - LOOKING AT NATURAL ANTIBIOTICS.

CAN YOU FIND NATURAL ANTIBIOTICS THAT COULD HELP TO OVERCOME THE PROBLEM OF ANTIBACTERIAL RESISTANCE?

Honey is a well known natural antibiotic. It contains hydrogen peroxide which may account for some of its anti-microbial properties. The high sugar content means a reduced water content and it is thought that the low water content is what limits microbial growth. If you have ever had honey and warm water when you have a sore throat that would be why. Check out the ANTIBIOTICS UNEARTHED project to help you find ways to discover and test new antibiotics **https://microbiologysociety.org/education-outreach/antibiotics-unearthed.html.**

1. Look for examples of other natural antibiotics both in and out of the kitchen. Research into the properties they have that would make them suitable candidates for bacterial treatment.

2. Try growing your own bacteria and testing your chosen natural antibiotics on the bacteria. To do this you will need to make some agar plates for your bacteria to grow. Try growing the bacteria *E.coli* onto the agar plates, then let it grow overnight at room temperature. To make your own homemade petri dish, check out this link **http://kitchenpantryscientist.com/microbial-zoos-homemade-petri-plates/**.

3. It is important that it is done under sterile conditions so your plates don't get contaminated. Make sure to use aseptic techniques (use a Bunsen burner flame or in a contained bacterial hood; wearing a lab coat; don't breathe or cough too close; tie long hair back; wash hands.) Make sure you tape up your plates so that anything that grows stays in the plate! **http://www.nuffieldfoundation.org/practical-biology**

4. Once you have tested your *E.coli* can grow, see if you can grow it on plates containing your natural antibiotics - include these in your agar, perhaps choose 3 or 4 natural antibiotics (honey and teatree oil are examples). **Can you observe any differences?**

5. To carry out this experiment you will need to do a risk assessment. Think about all the risks that could occur during the experiment and ways that you will limit the risk. You can use CLEAPSS HazCards at **http://science.cleapss.org.uk/Resources/HazCards/** to help you work out what the hazards are. Ensure you perform your risk assessment before you carry out your experiment. Dispose of any cultured bacteria appropriately and spray anything contaminated with bacteria using kitchen disinfectant spray!

6. You could test the antibiotics in combinations, do you see a larger effect on the bacteria? You could also test the experiment in different conditions (e.g. temperature) - **Does this affect the activity of the bacteria?**

A WEB-BASED RESEARCH PROJECT - BIOINFORMATICS OF RESISTANT BACTERIAL STRAINS

BIOINFORMATICS IS A SCIENCE THAT COLLECTS AND ANALYSES BIOLOGICAL DATA TO DETERMINE PATTERNS AND GAIN MORE INFORMATION FROM THE RAW DATA.
Use bioinformatics to determine relationships between resistant microbes - do they have the same genes and are these genes found to be commonly mutated?

1. Start an internet search to identify some drug-resistant pathogenic bacteria. One example is *Mycobacterium tuberculosis* (TB). Write up everything you can find out about the bacteria including appearance, disease, history of antibiotic treatment and the mechanism of resistance. Include facts, diagrams and figures. Determine the mechanism by which the bacteria has gained its resistance, look for specific proteins involved in this resistance. You should also make a list of the websites and references you used to make this literature review.

2. The next step is to find the genome DNA sequence. This is known as the DNA FASTA sequence that gives you the genetic code in the four bases A, C, G and T. You will want to choose one gene to look at. Choose your gene based on your literature review, perhaps the gene involved in resistance. So going back to the TB example, choose the RNA polymerase subunit beta gene (rpoB). Mutations in this gene leads to resistance to rifampicin, an antibiotic, resulting in high-resistance to the drug. Each bacterium may have several resistance genes but just choose one to start with. Think about why you are choosing this gene and justify it in your lab report.

3. Look up the sequence of this gene, (the FASTA format of A,T,G,C letters) at EnsemblBacteria (**bacteria.ensembl.org**). Type in the bacterium species in the genome box and press go. A list will appear and it will usually be the first one. For example, type in *Mycobacterium tuberculosis,* click the first result and it takes you to a page with further information about the genome. Then you need to search for your specific gene in the bacterium genome and press go e.g. rpoB. Click on the result gene ID and this will give you lots of information about the gene - the location on the chromosome, the size of the gene, the protein it makes - write this all down for your report. A table down the left side of the page will have different tabs. Click on the sequence tag and a FASTA sequence should appear. Copy and paste this sequence into a word document. Try the other tags to see what other information there is.

4. The next step is to do the same for other bacteria looking at the same gene. You could stick to the same bacterium genus, for example you could next look at *Mycobacterium leprae* or *Mycobacterium bovis*. Look at what diseases, if any, these bacteria cause.

MORE STEPS OVER PAGE

A WEB-BASED RESEARCH PROJECT - BIOINFORMATICS OF RESISTANT BACTERIAL STRAINS

CONTINUED...

5. Once you have all the DNA sequences - 4 or 5, choose 3 from the same genus and 2 from a different one - you want to compare the sequences. Use EMBOSS Needle Pairwise sequence alignment (**www.ebi.ac.uk/tools**) to do this. Compare each sequence to your original sequence of choice, so you should have five comparisons. The fill in page looks daunting but just insert your FASTA sequences into the two boxes and press go! When the results come back the software will have aligned the two sequences, where the sequence is the same there will be a line between the letters, save the file of the alignment and note down the % similarity. So with my example, *M.tuberculosis* and *M.Leprae RpoB*, genes are 82.3% similar. Create a table of the similarity percentages.

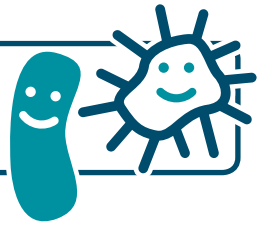
6. Now you want to build a phylogenetic tree to be able to visualise the similarity of the genes between the species. There are a few tools you can use to do this and here is your chance to try some out for yourself. Use **https://molbiol-tools.ca/Phylogeny.htm** to start. A more simple one is **http://www.phylogeny.fr/simple_phylogeny.cgi**. Insert all the sequences into the box (see example insert to show you how to do this) and press go. This then produces a phylogenetic tree. Perhaps try a few other sites to see if your trees look the same.

7. To take this further, you could locate the high-resistance mutation points in the sequences of your chosen genes and see if they match across the sequences. Think about how these mutations affect the overall sequence and the effect it may have on the protein.

8. Try thinking about what this has shown you and how the similarity may result in resistance spreading. **How can this genetic information help to tackle antibiotic resistance?**

A COMBINED PROJECT - INVESTIGATING PROBIOTICS

ARE PROBIOTICS A GOOD WAY TO PREVENT BACTERIAL INFECTION? INVESTIGATE WHETHER THE 'FRIENDLY' BACTERIA ARE JUST A MYTH.



1. Probiotics are live bacteria and yeast that have been previously shown to have health benefits. They are usually referred to as the 'friendly' bacteria. Probiotics have been added to a lot of yoghurts and other foods and even taken as food supplements to restore the bacteria in your gut. This article gives an idea of where they are found **https://www.healthline.com/nutrition/11-super-healthy-probiotic-foods#section1**.

2. A few issues have arisen around the use of probiotics, can you identify what these are? A lot of new data points to them being ineffective and not useful to human health. Look at this link showing a recent study by the NHS suggesting they have no effect: **https://www.nhs.uk/news/food-and-diet/no-evidence-probiotics-promote-gut-diversity-in-healthy-adults/**. However, research into microflora transplants have shown to be highly successful; these are prebiotics. What are the differences between probiotics and prebiotics? What is the evidence that has been shown for them being effective and ineffective, can you draw any conclusions from your findings?

3. Carry out a study on the probiotics found in your local supermarket foods - start in the yoghurt and milk section as there will be plenty there. What do the labels say, do they support these products with any scientific evidence of their effects? Can you find them elsewhere; there has been a growth in 'breakfast on the go' products, this may also be a good place to look.

4. Analyse the benefits of including these bacteria in our diet and whether they work. Do an extensive literature search to determine whether these bacteria are necessary and determine the benefits of these in our body. Summarise your findings by making a recommendation of your own on the foods we should eat and the foods we shouldn't eat that contain probiotics. There is a lot of conflicting research so look at the different arguments and determine what you think based on the evidence provided to you.

EXAMPLE PROJECT IDEAS

A WEB-BASED RESEARCH PROJECT - USING PREBIOTICS TO OPTIMISE GROWTH

TESTING DIFFERENT PREBIOTICS TO ASSESS THE BENEFICIAL GROWTH TO BACTERIA.

1. As discussed previously, prebiotics are compounds that are used to direct the gut microbiota to benefit the host (the human). There are natural prebiotics that include raw garlic and leek and usually contain fibre. Not much is known about the mechanism or properties of prebiotics making their relevance greatly questioned by scientists.

2. Carry out a study to determine resources that allow bacteria to grow better, much like the activity of the prebiotics in the gut. Choose a range of natural prebiotics, including garlic and leeks, and analyse the effect of these on growing *E.coli*.

3. You will need to grow *E.coli* on agar medium containing these different prebiotics. Make sure to plan your experiment and carry out a risk assessment for the experiment and resources you are using. Ensure you dispose of the bacteria appropriately and sterilise any bacteria using kitchen disinfectant spray. Grow your bacteria in the different prebiotic containing medium at room temperature overnight. Assess how the bacteria has grown. **What prebiotics work and which do not?**

4. Carry out an extensive study on prebiotics - what they are, where they are found etc. A good website to look at first is **https://www.bimuno.com/prebiotics**, then perhaps looking more specifically at these areas. Look at current research being done on prebiotics; you could look at the news for this. You can always look at the references on Wikipedia as a good place to start. **What is known about how they work and what is still unknown? How do they compare to probiotics? Look at the sources of your resources, are they from research labs? Can you trust this data?**

5. Look more into the microbiome. Prebiotics are used to ensure we have a healthy gut for digestion, but what other implications are there associated with the microbiome? (**https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3917468/**). Animal studies have recently implicated the human gut in anxiety and mental health. What can you find out about this and what research is being done to help mental health using the gut microbiome?

EXAMPLE PROJECT IDEAS

AN INTERACTIVE RESEARCH BASED PROJECT - ACTION PLAN, STOP A PANDEMIC OUTBREAK!

PROPOSE A PLAN TO THE GOVERNMENT ON HOW TO OVERCOME ANTIBIOTIC RESISTANCE.
The government need some scientific advice on how they would deal with an antibacterial resistance outbreak. The government are looking for a detailed report with supporting evidence and a presentation of your proposal.

1. Start by choosing your resistant bacteria. Do some research on resistant bacteria - a few examples you could look at include diseases caused by bacteria such as pneumonia, meningitis, multidrug-resistant *Mycobacterium tuberculosis* and MRSA (methicillin-resistant *Staphylococcus aureus*). Pick a resistant bacterial strain to focus on.

2. Next, do some research about how that bacteria has developed resistance, look at how the bacteria has changed on a genetic level and what genes have been mutated that give rise to resistance to different antibiotics. Look at changes that occur to both its structure and function. Within your report, use examples and case studies of how the bacteria have shown resistance - any epidemics, new outbreaks or reports of new resistance - old newspapers would be a good place to look for this.

3. Make some 3D models (use clay or paper) or drawings of your bacteria to show the resistance changes. For example, a mutation in a structure gene that prevents the antibiotic recognising the bacteria could be portrayed as a model. Think about how these bacteria make people sick, their incubation time within a host as well as their infection time. Gather as much information about your bacteria as possible. **This is your superbug.**

4. Look at antibiotics that currently treat your bacteria, visualise how they work, do they kill the bacteria or slow its growth? Perhaps make some models or diagrams to show how it interacts with the current strain and how it can't interact with your superbug.

5. **Outbreak!** Pick a place in the world where your outbreak begins. Make sure you think about why you are choosing this location; is this an area where the disease is prevalent, perhaps an over-crowded city? Assess how your superbug would spread from the source by looking at popular travelling routes by sea and by air, determine how your superbug would be carried - for example the plague was carried by rats. Make some predictions about how long it would take to spread worldwide, and create a map of how this would happen. Remember to justify your predictions.

6. **Your Solution!** Assess how to maintain the infection and limit spread. Think about setting up quarantines and methods of keeping the public calm. Use evidence from previous disease outbreaks. Design a new antibiotic but be sure to think about the time scale and methods you would use to achieve this and then the distribution.

HEALTH AND SAFETY.

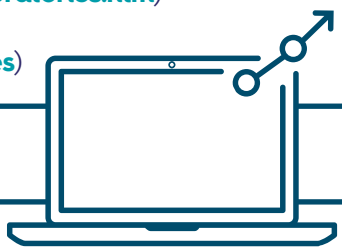
Based on guidelines from the Health and Safety Executive (HSE), anyone who works with microbial agents in microbiology laboratories is at risk of potential exposure to biological agents, be it through direct work with biological agents themselves (e.g. microbial cultures) or through contact with materials that may contain microorganisms (e.g. blood). The specific safety measures to be implemented in laboratories will heavily depend on the biological agent being used and its correspondent hazard, with biological agents being classified according to 1 (the lowest) to 4 (the highest) scale depending on their ability to infect healthy adults, where 4 would be necessary for work with extremely dangerous pathogens such as the Ebola virus.

All activities involving work with microorganisms are controlled by the Control of Substances Hazardous to Health (COSHH) Regulations, with teachers and technicians having a duty under the Health and Safety at Work Act to comply with any safety instructions given by their employers, including the use of model risk assessments referring to appropriate publications such as: *CLEAPPS Laboratory Handbook* (2006), section 15.2; *Topics in Safety*, 3rd edition (ASE 2001); *Microbiology: an HMI Guide* (DES, 1990); and *Safety in Science Education* (DfEE, 1996). These guidelines are essentially common sense and do not represent an obstacle to the conduction of microbiological research in school laboratories.

Areas for consideration when planning to embark on practical microbiology investigations should include:

- Preparation and sterilization of equipment and culture media
- Preparation of microbial cultures as stock culture for future investigations and inoculum for the current investigation
- Inoculation of the media with the prepared culture
- Incubation of cultures and sampling during growth
- Sterilization and safe disposal of all cultures and decontamination of all contaminated equipment

Source: The Health and Safety Executive (**http://www.hse.gov.uk/biosafety/laboratories.htm**) and the Microbiology Society's Safety Guidelines (**https://microbiologyonline.org/teachers/safety-information/safety-guidelines**)



USEFUL LINKS

CAUSES OF ANTIBIOTIC RESISTANCE
www.who.int/drugresistance
www.who.int/antimicrobial-resistance/en/

THE CENTRE FOR DISEASE CONTROL AND PREVENTION INFORMATION
www.cdc.gov/drugresistance/index.html

BECOME AN ANTIBIOTIC GUARDIAN
www.antibioticguardian.com

PLAY SOME GAMES ON ANTIBIOTIC RESISTANCE
https://www.shu.ac.uk/research/specialisms/biomolecular-sciences-research-centre/news/weve-created-a-bacteria-builder-online-game

Author: Tabitha Jenkins, March 2019