THE TRAGIC CASE OF STAN: Extended reading
Diagnosing infections

Determining the microbial cause of an illness can be a key to successfully treating the disease. This is because different infections often lead to similar symptoms but require completely different treatments. For example, treating a viral infection with antibiotics will not kill the pathogen, nor will treating malaria (a disease caused by an eukaryotic parasite) with antibiotics or antiviral drugs. Hence, we need to be able to identify what is causing an infection.

How would you distinguish between two different bacteria that cause the same symptoms?

You wake up one morning with a scratchy throat. Your muscles feel weak and you can feel a fever coming on. Most infectious diseases have some common symptoms including fever, weakness, coughing, and more. So, how can you determine which infectious agent is causing your symptoms? With so many possibilities, sometimes the process of elimination is more powerful than direct testing for the presence of a specific pathogen.

Think back to Unit One. We learned that many bacteria have specific structures that help them cause disease or evade the immune system. Many of those structures make the bacteria look drastically different from each other under a microscope, in addition to their general appearances such as rods, spheres, or spirals.

Bacteria have three major morphologies (physical forms)

- Cocci (spheres)
- Bacilli (rod-like)
- Spirals
However, being able to identify the morphology of a bacterium raises yet another challenge, for instance, how could you tell the difference between two spherical bacteria?

**Gram staining**

Gram staining is often used as one of the first tests to identify bacteria both in research and as a clinical diagnostic. Gram staining, on its own does not identify the infectious agent but is an important starting point because it divides bacteria into two major classes, Gram-positive and Gram-negative.

Gram positive bacteria will absorb the blue/purple crystal violet and the iodine complex in their cell walls, and keep it there during the decolorizing step.

Gram negative bacteria will lose the crystal violet and iodine complex during the decolorizing step. Remember that the Gram-negative bacteria have an outer membrane rich in lipids and this can be damaged by the alcohol (see Lesson 1.3 for more details).

Knowing that the morphology of an infectious agent is Gram-positive cocci tells us that the infection is not caused by Gram-negative pathogen such as *E. coli*. Even though this information does not identify the pathogen, it could be important for treatment — remember Gram-negative bacteria are more resistant to antibiotics compared to Gram-positive bacteria.

However, this information is only the first step in the process. You will still need to collect more data to allow you to make an educated guess about the identity of the pathogen, so the process of elimination continues.

As you remember from lesson 1.3, there are other bacterial structures and these can be used for identification. Flagella is a perfect example.
Flagella can provide a rather conspicuous clue to help you decide whether two similar-looking bacteria are the same. For example, here are images of *Vibrio cholerae* and *Salmonella typhi*. Both are Gram-negative rod shaped bacteria. Once you have a candidate, antibodies can be used for positive identification. How will that work?

When we are exposed to a pathogen, our immune system often creates antibodies as a specialized defense. The antibodies will attach tightly to a specific pathogen using a lock and key mechanism (we will examine how in detail in Unit 5). These antibodies can be purified and used in the lab for pathogen identification. For example, a patient with severe watery diarrhea who has swimming bacteria in his stool sample when observed under a microscope. The symptoms resemble cholera and you have antibodies that bind to the *V. cholerae* flagellum. These antibodies are heavy molecules, and when many of them bind to the flagella, they will prevent them from moving, and the bacteria will stop swimming. So, when you add them to the sample, and look again under the microscope, you will see that the bacteria are not swimming anymore. These antibodies provided an opportunity to positively identify a pathogen without ever isolating it.

**Using the process of elimination to isolate a microbe**

In Stan’s lab case, you will first plate Stan’s skin sample on a Nutrient agar (NA) plate. It was beige in color and it is called all purpose growth media because it is made from a rich nutrient mixture. The two most common all purpose medias are Luria-Bertani broth (LB) or Nutrient broth (NB). They can be made solid with the addition of agar making them LBA (Luria-Bertani agar) or NA (nutrient agar). They are both media rich in nutrients and contain:

- Peptides and amino acids
- Sugars
- Lipids
- Vitamins (including B vitamins)
- Trace elements (e.g. magnesium, calcium)
- Minerals like sodium chloride
We use a nutrient rich plate, so we will be able to grow as many different types of bacteria as possible. Let’s say that you Gram stain colonies that grew on the NA and observed them under a microscope. You found out that they were spherical Gram-positive bacteria (cocci). Since you isolated them from Stan’s skin, you may suspect that they are a *Staph.* species since they are Gram-positive cocci and naturally thrive on skin. How could you further narrow down the species, whether it is regular *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) or even *Staphylococcus epidermidis*? The answer is critical because each of them will need a very different antibiotic treatment in case of an infection. For this purpose, scientists have designed selective-differential media.

You will plate Stan’s skin sample on a selective-differential medium, called mannitol salt agar (MSA), and in order to save time you did as early as Day One.

MSA is red in color because it contains a pH indicator called pehnol red. It is red under neutral pH but changes to yellow under acidic conditions. The medium also contains mannitol (a sugar) and a higher concentration of salt in addition to the nutrients that are present in the LB plates. This high salt concentration encourages the growth of some bacteria like *Staphylococci* while inhibiting the growth of others, making these plates selective for bacteria that can thrive under high salt such as skin bacteria. The mannitol in the plates has its own purpose — to differentiate between different *Staph.* species. If bacteria growing on the MSA plate can ferment the mannitol they will convert it to acids. The acids will cause the phenol red to change color to yellow. This is diagnostic for *Staphylococcus aureus* (see image above). Although several different species of *Staphylococcus* can grow in the nose or skin, for instance *Staphylococcus epidermidis* and *Staphylococcus aureus*, only *S. aureus* can ferment mannitol and turn the red indicator color yellow. So, this reaction allows us to distinguish between *S. aureus* and other *Staph.* bacteria.

Why is this important? Some *S. aureus* bacteria called MRSA (methicillin-resistant *S. aureus*) have mutated to become extremely drug resistant.
BACKGROUND

The presence of MRSA means that careful precautions must be taken to prevent spread of the bacteria to other people, because if infection occurs it is very hard to treat. About 1% of the population have MRSA in their nose as part of their commensal bacteria without apparently suffering any effects. However, these people should be very careful with hygiene to avoid infecting others who may be more susceptible, especially in a hospital setting.

Antibiotic resistance

When you get sick with a bacterial infection, a doctor can prescribe you an antibiotic that will probably help you get better. However, we are fast entering a world where many pathogens are resistant to many of the antibiotics in our arsenal, as is the case with MRSA (image on the right). Here, we will address some social and medical problems associated with antibiotic resistance. We will also explore the dangers, causes, and potential solutions to this important problem.

Why are we worried about antibiotic resistance?

Treating bacterial infections with antibiotics is becoming less successful because many bacteria have adapted mechanisms that prevent their normal susceptibility to antibiotics. This poses a threat that is much more serious than you may be aware of. Antibiotics are used during surgery, when needed during pregnancy, trauma, age or disease related immune failure, and much more! You may have read headlines in the news talking about MRSA outbreaks in hospitals. As we began to explore in the Lab Case, MRSA is the name given to the Methicillin-resistant form of the common bacterium Staphylococcus aureus. More recent forms of MRSA are resistant to multiple antibiotics that have been commonly used to treat Staph. infections. These new bacteria that are resistant to many antibiotics are called also superbugs. MRSA infections are a major concern in hospitals, where there are numerous people with compromised immune systems as a result of disease or trauma.

Unfortunately, MRSA is not the only superbug. The number of superbugs has been increasing steadily in the past couple decades.
**BACKGROUND**

**Growing concerns: the return of tuberculosis (TB)**

Tuberculosis is an infectious disease caused by a bacterium called *Mycobacterium tuberculosis*. It is one of the most ancient diseases and was once the leading cause of death in the US, and many European countries. Unfortunately, it is still the leading cause of death in the poorest regions in the world. For most of the 20th century, tuberculosis in the developed world was declining. Much of this decrease was attributable to the effective use of antibiotics. However, recent years have seen a global increase in the incidence of multiple drug resistant tuberculosis or MDR-TB, which is resistant to two or more of the major front-line drugs used to treat tuberculosis infections. MDR-TB poses a major health concern because we are quickly running out of new drugs to treat and cure tuberculosis.

Even more worrying is the development of XDR-TB or extensively drug-resistant tuberculosis, which is resistant not only to the front line drugs, but also to the second-line drugs.

Patients infected with XDR-TB have only about 30% chance of clearing the infection and are at serious risk for death.

**How did we get here?**

**Misuse of antibiotics is a major factor in building resistance.**

Today, we give antibiotics to livestock to increase their growth rate, and we also use antibiotics in cosmetics. In addition, prescribing antibiotics before identifying the microbial origin of an infection increases the odds of giving antibiotics to people who are infected with viruses — a futile response that again encourages antibiotic resistance. Many bacterial and viral infections have symptoms that are nearly identical. For example, it is very difficult, if not impossible, to distinguish between Norovirus (a virus that causes stomach flu) and *Salmonella* (bacterium that causes gastrointestinal infection), when considering the symptoms alone.
Both have symptoms of nausea, vomiting, diarrhea, abdominal pain, headache, and lethargy. Norovirus infections accounts for about 50% of ‘food poisoning’ cases in the United States. It can be passed from person to person, as well as through food or water that is contaminated by feces. Norovirus infection is seldom fatal but highly contagious. Since it is caused by a virus, antibiotics will not treat it. *Salmonella*, on the other hand, is a bacterium that can be contracted from contaminated food or water, or from exposure to reptiles. In rare cases, it can result in severe dehydration that leads to death.

What do you do when faced with a set of symptoms that could be the result of a bacterial infection OR a viral infection? How can you weigh the costs and benefits of a treatment? Some infections can be easily diagnosed, such as Strep throat, but others require timely and expensive procedures. For minor illnesses, the most common way is to wait and see. Most viral infections are cleared, or at minimum subsided, in a few days, so if you are still sick after that time it may be justified to pull out the antibiotic.

**How bacteria become antibiotic resistant?**

In Unit 1 and 3, we learned how microbes adapt their structures in response to challenges, for example some bacteria change their flagella proteins to evade detection by the immune system. Likewise, microbes can adapt, following the challenge of antibiotic treatment.

Under optimal growth conditions, which the human body can offer, bacteria grow and divide into high numbers pretty quickly. But bacteria not only grow and divide quickly, they also accumulate changes in their genetic material.

When antibiotics are used to treat an infection caused by billions of bacterial cells from the same species, there will be at least a few bacterial cells that have randomly mutated and developed adaptations that protect them from the antibiotic. Then, through the process of natural selection, when the bacteria susceptible to the antibiotic are killed off, the resistant bacteria will have all the resources to themselves. They will divide and grow into huge numbers, and afterwards spread to other hosts, animals, etc.

**Hence, every time we use antibiotics we are encouraging bacteria to adapt to resist them.**
How do you know which antibiotic to use?

Not all antibiotics work in exactly the same way, so it is sometimes difficult to know whether an infection is resistant to an antibiotic because it is caused by a resistant bacterium or by a virus that wouldn't respond to antibiotics.

The most rigorous way to decide which antibiotic to use, is to isolate the bacteria and then test for antibiotic susceptibility, like we will do in the lab. Unfortunately, this is not always an option because pathogens can be difficult to isolate, as we have discussed before. In those cases, doctors will often use trial and error: try one antibiotic for a while, then change to another if it doesn't work, or use cocktails of antibiotics at once. As we will see in the Stan, this can be high stakes gamble.

A summary of measures that can limit antibiotic resistance:

- Take the full course of medication.
- Avoid low doses for extended times which will give the bacteria more time to accumulate mutations that will make them antibiotic resistant.
- Use antibiotics only for serious infections.
How are we going to solve the antibiotic resistance crisis?

The search for new antibiotics is not over, although going at slower pace for numerous reasons. Most antibiotics are produced by soil bacteria and fungi but it is getting harder to discover novel antibiotics made by these microbial friends. So, scientists are now trying to look for antibiotic-producing organisms in previously unexplored environments such as the oceans or use novel techniques with the old producers.

Using the bacteria’s enemy in nature

Bacteriophages were discovered in the early 20th century and were originally considered as therapeutics but antibiotics proved effective and quite easy to produce in large quantities, thus becoming the primary therapy. In the past decade, there has been serious research effort to tap the potential of bacteriophages as an alternative treatment agents of infectious diseases.

Phages have the potential to be the best alternative to antibiotics. Since they have high specificity, one phage infects only one bacterial species, so they will not wipe out the beneficial bacteria in our bodies together with the pathogen, as is the case with antibiotics. Viruses, in general, are considered the most numerous organisms on our planet. Estimations have that number as high as $10^{31}$ viral particles.
BACKGROUND

This potential seems endless! However, since phages contain their own molecules, such as proteins, that are foreign to our immune system, they are cleared pretty quickly from our bodies. Maintaining the phage particles in high enough numbers, to achieve complete clearance of an infection, is one of the major hurdles that needs to be overcome.

Other options?

Another option is looking for novel molecules, possibly of bacterial origin, that function in a different mechanism compared to antibiotics. This will potentially limit the development of resistance to antibiotics. One example is finding signal molecules that are responsible for the successful communication among bacterial cells in a community, and interfere with their function interrupting the normal communication (for more information watch Prof. Bessler’s talk in Lesson 3.1).

Antibiotics use comes at additional cost for our bodies.

When antibiotics are prescribed to eliminate pathogens from our bodies, they also wipe out bacteria that are part of our natural microbiota. We are just beginning to learn the key role that our microbiota plays in our overall health. Our microbiota seems to be associated with a number of different conditions including obesity, diabetes, metabolic syndrome, and allergies. This research field is relatively new and establishing causation is quite challenging, e.g. proving that one or a few bacterial species are directly causing any of the forementioned conditions. Nevertheless, we have learned that the microbial diversity in our bodies, that is unique for each individual, is significantly altered as a result of antibiotic treatment. These changes can have short-term, and possibly long-term, health consequences.

The nightmare of C. diff. infections

In the lab case, Stan is treated with many different antibiotics and spent some time in a hospital. These are both risk factors to developing a Clostridium difficile infection (CDI).

What is C. diff?

C. diff is a bacterium that is found in many habitats in nature such as soil, water, air, gut, and feces of animals and humans. In fact, the species name comes from the Latin difficulte meaning “difficult, obstinate”. Some people harbor C. diff in their guts but experience no ill symptoms most likely because the bacteria numbers are kept in check by the rest of the microbiota (your other gut bacteria).
**BACKGROUND**

*C. diff.* cells are Gram-positive rods. It has a couple characteristics that contribute to its pathogenicity.

First, it produces toxins which cause most of the symptoms associated with CDI such as severe diarrhea, and life-threatening inflammation of the colon. **The tool that makes *C. diff.* so successful, in spreading and surviving - is its ability to form spores.**

The spores will not be affected by a course of antibiotics since antibiotics target bacterial cells. Antibiotics treatment will either suppress the growth of many gut bacteria or kill them leaving unoccupied niche and available resources. This presents a great opportunity for the *C. diff.* spores lurking in the gut. They will germinate and start growing much faster than the other bacteria.

Unfortunately, they will also produce toxins making the host sick. The spores can also be shed into the feces and in the case of inadequate hygiene in hospital or community settings can be transferred to other people. **Not everyone who gets infected with the spores will get ill. But people who are immunocompromised and/or undergoing antibiotics treatment are at higher risk.**

**CDI are now the leading hospital associated infections in the USA. It can be quite difficult to treat with antibiotics, since it has high recurrence rates.** This means that even though the infection may initially subside after antibiotic treatment, it often comes back.

The diarrhea and the bacterial toxins can leave patients severely dehydrated, and can damage parts of the colon. CDI carries risks of complications such as the need to remove the whole colon or part of it.

The mortality rate of CDI ranges from about 4% to almost 17%. Other alarming facts are the appearance of new and more dangerous *C. diff.* strains, and the increasing number of infections in community settings among people who are not considered at high risk for *C. diff.*
How *C. difficile* Spreads.

George, a 68-year-old man, goes to the doctor’s office and is diagnosed with pneumonia. He is prescribed antibiotics, drugs that put him at risk for *C. difficile* infection for several months.

One Month Later

George breaks his leg and goes to a hospital. A healthcare worker spreads *C. difficile* to him after forgetting to wear gloves when treating a *C. difficile* infected patient in the next room.

Hospital

Doctor’s Office

Rehab Facility

Two Days Later

George transfers to a rehabilitation facility for his leg and gets diarrhea. He is not tested for *C. difficile*. The healthcare worker doesn’t wear gloves and infects other patients.

Three Days Later

George goes back to the hospital for treatment of diarrhea and tests positive for *C. difficile*. He is started on specific antibiotics to treat it. Healthcare workers wear gloves and do not spread *C. difficile*. George recovers.

**Source:** CDC, 2012
When antibiotics seem to be the problem.

As the number of *C. diff* infections increased, as well as the challenges in treating them, scientists and doctors started to look for new treatments. Since one of the main issues leading to CDI seems to be the disturbance in the normal gut bacteria that keep *C. diff* growth in check, treatments to directly replace the missing microbes are a hot topic. **This therapy was called fecal transplant or fecal bacteriotherapy.**

In the 1950s, fecal transplant was first used to treat four patients with colon inflammation. All of them recovered within hours after the treatment was applied. The first report for fecal transplant to treat CDI was published in the 1980s. Since then, there have been more than 500 documented cases with an average success rate of about 95% resulting in complete recovery. The resolving of symptoms is quick, often times within hours after the treatment is applied, much faster compared to antibiotics!

Fecal bacteriotherapy is still considered an experimental treatment but that might change soon. Fecal bacteriotherapy is a promising treatment uniting doctors, scientists, and regulators in their efforts to make this therapy a standard of care. Overall, the process needs better standardization, especially in regard to the fecal material; there is always a risk that donors may be harboring bacteria that are harmless to them but can be deadly to a recipient (remember Typhoid Mary from Lesson 2.1), who is already in fragile health. Initially donors were considered on a patient by patient basis. Healthy people who live in the same household with the recipient were considered the best candidates but this is often quite challenging. Recently some studies showed that the relatedness between the donor and the recipient is not a significant factor in the success of the procedure. But since our knowledge of the importance of the human microbiota in a person’s overall health has been increasing, it seems important that donors are in overall good health especially in regard to their gastrointestinal condition. **Scientists are now working to develop methods to assure the safety and effectiveness of standardized fecal material that can be readily available to all patients with CDI.** Fecal bacteriotherapy is now also being considered for clinical trials to treat many other conditions among which diabetes, metabolic syndrome, and even autism.
Sources used

http://www.cdc.gov/HAI/organisms/cdiff/Cdiff_infect.html

http://www.mayoclinic.org/diseases-conditions/c-difficile/basics/definition/con-20029664

