Evolution, Antibiotics, and Us

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Antibiotics are an integral part of modern medicine, so widely used that healthcare without them has become inconceivable. But have you ever wondered what antibiotics look like from the perspective of bacteria? How antibiotic treatment changes their environment? How bacteria adapt to this dramatic change? And what happens to the "good" bacteria in our body during treatment? In this issue, we will make an excursion into the fascinating world of bacteria and have a closer look at the evolution of antibiotic resistance.

Before the era of antibiotics in which we live today, many diseases were nearly untreatable. Even small cuts, if they became infected, could lead to amputated limbs, or even death. Bacteria—the cause of these infections and of many diseases such as bacterial pneumonia or tuberculosis—are single-celled organisms. They reproduce asexually: a bacterium divides into two daughter cells that are almost exact copies of the original, up to a few mutations. How fast bacteria divide depends on both the species and the environment, i.e. the site of infection. The division time of bacteria in the human body is on the order of hours. Thus, a few days (or even just one) are often sufficient for bacteria to reach a population size large enough to make us ill.

In the second half of the 19th century, several researchers made an intriguing discovery.

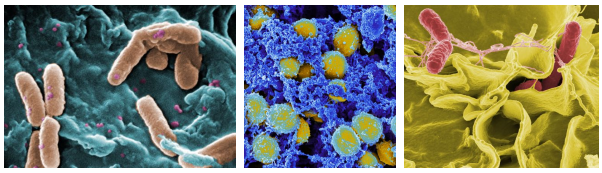

Fig. 1: Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella species.

Images: (1) Centers for Disease Control and Prevention/Janice Haney Carr, (2)+(3) National Institute of Allergy and Infectious Diseases.

Laboratory animals injected with both *pathogenic* (i.e. disease-causing) and certain other types of bacteria would not develop anthrax or cholera (two dangerous infectious diseases normally caused by the injected pathogenic bacteria).

One of these protective bacteria was *Pseudomonas aeruginosa* (at the time, called *Bacillus pyocyaneus*), and it turned out that it secreted a substance that suppressed the growth of several pathogens and could be used to treat the associated diseases. This secretion, known as pyocynase, became the first antibiotic ever used in a hospital! The real breakthrough, however, was achieved with the development of penicillin, which was not only very effective but also had no or only weak toxic effects.

However, Alexander Fleming (one of the "fathers" of penicillin) soon realised the risk of bacteria becoming resistant to penicillin, a phenomenon he had observed in the lab and in patients. Resistant bacteria—unlike their sensitive relatives—can multiply and grow in

spite of the presence of the antibiotic. The specific drug is thus ineffective, and cannot be used to treat the infection. In an interview with the New York Times in 1945, Alexander Fleming warned:

"In such a case [self-medication with too-small doses] the thoughtless person playing with penicillin treatment is morally responsible for the death of the man who finally succumbs to infection with the penicillin-resistant organism. I hope this evil can be averted."

Today, antibiotic resistance has become one of the greatest dangers to health care worldwide. The World Health Organisation (WHO) states that resistant bacteria are responsible for 25,000 deaths every year in the European Union alone.

Where do these resistant bacteria come from? What makes them different? And what does evolution have to do with it? These are some of the questions we will explore in the following sections.

The discovery of penicillin: a tale of chance and people

Before Alexander Fleming left St. Mary's Hospital in London for his summer vacation in 1928, he left some petri dishes with *Staphylococci* bacteria on his lab bench. When he came back in September, either he or his colleague Merlin Preyce—history is vague on this point—spotted something interesting: One of the plates had been contaminated by a mould, and around this mould, there was a bacteria-free zone! Fleming said "That's funny." And with these words, one of the most influential discoveries in the history of medicine began. Further investigation revealed that the mould belonged to the bread mould family *penicillium*, and it secreted a substance that could kill bacteria of various species.

Alexander Fleming preserved some of this specific mould and wrote an article about his findings. This article was found ten years later by Ernst Chain, who was a researcher in Howard Florey's team at Oxford University. This team began testing the secretion for use in medical treatment. One of the major obstacles turned out to be how to purify enough of the antibiotic substance. The solution to this problem is largely due to Norman Heatley, another member of Florey's lab. It still took enormous efforts before Penicillin finally arrived on the market. Its use became widespread in the early 1940s, and saved millions of lives during World War II.

Only a few years later, in 1945, Alexander Fleming, Howard Florey, and Ernst Chain were awarded the Nobel Prize in Physiology or Medicine.

Antibiotic resistance

Let us consider one example of how an antibiotic works and how a cell can become resistant to it. Before a cell divides, its DNA needs to be replicated, and the cell possesses a whole toolkit of molecules for this purpose. When our antibiotic agent enters the cell, it attaches itself to one of these tools, preventing the tool from working properly. Instead of performing its task in DNA replication, the tool destroys the DNA! We have achieved our goal: the cell dies. But among the many bacteria, there may be some with a mutation in their DNA that causes the tool to have a slightly different shape. In this case, the antibiotic cannot attach to it, like a key that does not fit a lock. Cells with this altered tool are not affected by the antibiotic and can replicate normally. They pass the life-saving mutation on to their daughter cells, which again replicate and leave descendants. After a while, all the normal cells are dead and the population consists entirely of mutated resistant cells. This is Darwin's natural selection among bacteria: the fittest ones thrive and take over. For us, this means that the infection is back.

There are other mechanisms through which a cell can be resistant to an antibiotic, for instance, a cell might be able to pump the antibiotic out faster than normal cells, or be able to destroy antibiotic molecules. The latter can occur inside or outside the cell. If sufficient amounts of the antibiotics are destroyed by resistant cells, even the neighbouring sensitive cells are protected and can survive as well!

How do antibiotics affect bacteria?

To understand how antibiotics attack bacteria, we need to briefly consider what a bacterium looks like and how it lives. The outermost layer of a bacterial cell is the cell wall. Under the cell wall, there is a cell membrane that regulates which molecules go in and out. Inside the cell, there is a whole machinery made up of molecules such as proteins that are responsible for the "work" inside the cell. A cell's DNA stores the information for the manufacturing of this machinery. When a cell divides, its DNA is replicated and distributed to the two daughter cells.
Antibiotics can attack bacteria in various ways. Some antibiotics destroy the cell wall of the bacteria or the cell membrane. Other antibiotics inhibit DNA replication or the production of proteins. A given antibiotic is not effective against all bacterial species; some bacterial species will have *intrinsic resistance*, and will not be affected by certain antibiotics. An antibiotic that is effective against many different bacterial species is called a *broad-spectrum antibiotic*.
When we are sick and take medication, antibiotics only do part of the job of clearing the infection. The rest is done by our immune system.

Let us take a closer look at the fitness of bacteria with and without the resistance mutation. As you know, the fitness of any organism depends on its environment. For a bacterium, an antibiotic can be a very significant component of its environment! Fig. 2 shows how the growth rate (i.e. the difference between the birth and death rates) of sensitive and resistant bacteria depends on the antibiotic concentration. The growth rate is one measure of bacterial fitness. You will notice that when the antibiotic concentration is close to zero, resistant cells actually grow more slowly than sensitive cells. This is because resistance mutations are often *costly*, i.e., cells with this mutation divide less often or die earlier than mutation-free cells. Thus, in the absence of antibiotics, natural selection tends to eliminate the resistant cells, and only few of them are present in the bacterial population.

In contrast, in the presence of sufficient antibiotic (starting at the left margin of the green region in Fig. 2), resistant bacteria grow faster than sensitive bacteria. As the concentration of antibiotic increases, the sensitive bacteria eventually may not be able to grow at all (the blue curve goes below the dotted line corresponding to no net growth). However, even resistant bacteria can only tolerate a certain amount of the antibiotic: if many antibiotic molecules attempt to attach to a mutated tool, chances are that one of them will succeed and break it. Thus at very high antibiotic concentrations (to the right of the green region), the resistant bacteria also show negative growth, i.e., they die out.

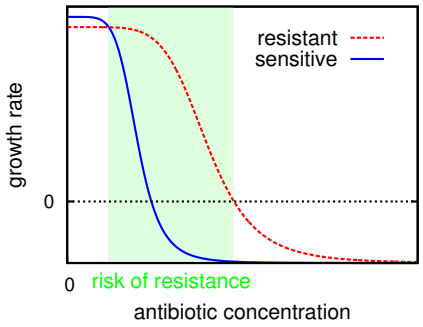

Fig. 2: Bacterial growth as a function of antibiotic concentration.

When bacteria are exposed to the concentrations of the green region in Fig. 2, we face the risk of resistance evolution: resistant bacteria are fitter than sensitive bacteria (the red curve is higher than the blue curve) and they are able to grow (the red curve is above the dotted zero line).

The level of antibiotic concentration a resistant cell can tolerate depends on the specific mutation. There are many mutations that confer resistance to low levels of antibiotics, whereas only a few make the cell resistant to high concentrations. Cells with several resistance mutations can withstand higher drug pressure than cells with just one mutation. For example, if the DNA that specifies the construction of the replication tool has two mutations, then the resulting tool would be altered to a greater extent, making it even harder for the antibiotic to attach to it.

Resistant bacteria may exist in the population prior to treatment or they can be generated first during treatment. Resistance is particularly likely if the drug dose is too low (remember Alexander Fleming's warning in the New York Times!). In order to prevent the evolution of resistance, one tries to give doses that are high enough that a single mutation in the DNA is not sufficient to achieve resistance. Acquiring two mutations that together provide resistance to these high doses, is much less likely than gaining one. If you think of rolling dice, getting two sixes is much less likely than getting just one six.

Another option is to administer two antibacterial drugs simultaneously. If a different mutation is required for each, the bacteria would need two mutations to survive. This strategy has been used to treat tuberculosis from very early on. In many other cases of bacterial infection, combination therapy is not applied. The reasons are diverse, and include an increased risk of side effects, higher monetary costs—or maybe also habit. Exploring the benefits and risks of combination therapy is an active field of research.

Where does selection for resistance occur?

Medicine: Whenever we take antibiotics (or give them to our pets), we promote the evolution of resistance. Today, antibiotics are used very widely, even though many everyday infections could be handled by our immune system on its own. Antibiotics are simply convenient because they speed up recovery. Antibiotics are even sometimes prescribed for viral infections, which is not only useless (antibiotics do not act against viruses!), but also imposes unnecessary selection pressure on our commensal bacteria (see below), and hence increases the probability that they will become resistant. In many countries, antibiotics can be bought without a prescription of a doctor.

Agriculture: Huge amounts of antibiotics are used in agriculture. Intensive livestock breeding requires high levels of treatment, and antibiotics are also used preventatively and as growth promoters (the latter is forbidden in the EU but it is, for example, allowed in the US). Agriculture is a serious driver of antibiotic resistance, and resistant bacteria from livestock can transition to humans through contaminated food, manure on fields, etc.

Environment: Antibiotics (and antibiotic resistant bacteria) are introduced to the environment through waste water from households, agriculture, and pharmaceutical companies. In one study, researchers followed the course of a river in Canada and found that the amount of antibiotics and resistant bacteria detected along the river matched the type of land use at these sites. Remember, even low levels of antibiotics that do not kill the sensitive bacteria may select for resistance.

Plasmids: vehicles of antibiotic resistance genes

Bacteria have a single chromosome that contains their genetic information. The essential genes that a bacterium needs, i.e. to grow, metabolize "food", and replicate, are all located on this chromosome. But in addition to the chromosome, bacterial cells can contain DNA in a second format called *plasmids*. Plasmids are (most often circular) DNA molecules that are separate from the chromosome and carry additional genes. During cell replication, they are also copied and transmitted to the daughter cells (just like chromosomal DNA). There are many different types of plasmids. No one really understands how or why these plasmids persist as they are not essential for the cell—in fact, they are often depicted as parasites (but read on!). And now, here is something really outlandish: some plasmids are able to transfer and "infect" a second cell, similar to a parasite transferring from one host to another (see Fig. 3).

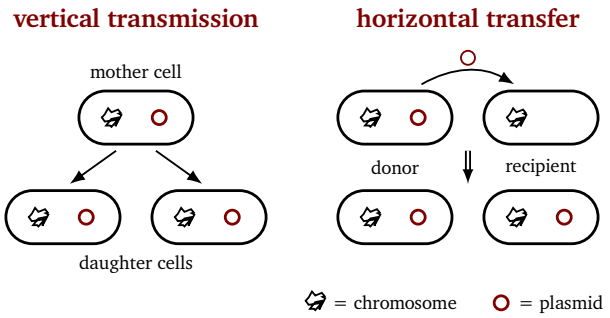

Fig. 3: The propagation of plasmids. On the left, you can see how a plasmid is passed from the mother cell to both daughter cells during cell division. The right side depicts the horizontal transfer of the plasmid: a copy of the plasmid is transferred from a plasmid-carrying to a plasmid-free cell. As a result, both cells carry the plasmid.

But plasmids have genes that influence traits. In other words, traits can be passed on from one cell to the other. To understand the impact of this, imagine you shake hands with your neighbor, and suddenly you have his eye color! Moreover, this transfer is possible between different bacterial species: imagine you stroke your cat, and you grow a tail! (Of course, the process of plasmid transfer is more complicated than shaking hands or stroking a cat.)

Back to antibiotic resistance: antibiotic resistance genes are often located on plasmids. For example, the plasmid can carry genes for "pumps" that transport the antibiotics out of the cell. Thus, a bacterial cell can become resistant not only by acquiring a mutation in the chromosome (as discussed above) but also by receiving a resistance plasmid. Resistance genes on plasmids can spread easily across bacterial communities, making their way from one species to another. This alone makes resistance on plasmids particularly dangerous, and what is more, plasmids often accumulate resistance genes to several antibiotics. If a cell receives such a plasmid, treatment with all of these antibiotics becomes ineffective. Thus, in the presence of antibiotics, such plasmids change from a burden into a great benefit for the bacterial cell.

Antibiotic resistance in commensal bacteria

Did you know that each of us carries approximately as many bacterial cells as our own human cells (of the order of 30 trillion)? These bacteria reside in our gut, on our skin, and in other body parts. We call them *commensal bacteria*. The word *commensal* derives from medieval Latin, where "commensalis" means "sharing a table". These bacteria are normally harmless or even beneficial inhabitants of our body, and interact with us in many complex ways that are far from being fully understood. For example, some of them help us digest food that we would otherwise be unable to process. They also protect us from pathogens: incoming pathogens need to compete for resources with our harmless commensal bacteria, making it harder for them to become abundant and cause an infection.

When we are sick and take antibiotics, we expose not only the pathogen (that we want to attack) but also part of our commensal flora—the community of our commensal bacteria—to the drug. This has two effects. First, by killing "good" bacteria, the flora is disturbed. As a consequence, the "bad", toxin-producing bacterium *Clostridium difficile* can become abundant and cause diarrhea, a common side effect of antibiotic treatment. Alterations of the commensal flora due to antibiotic therapy might persist for years, and may have unknown long-term consequences. Some researchers have suggested a link to an increased risk of certain diseases such as Crohn's Disease. Second, the antibiotics select for resistance in the commensals: drug sensitive commensals are killed, and drug resistant commensals spread. In fact, even without recent antibiotic treatment, we are very likely to harbor antibiotic resistant bacteria in our commensal flora, which we may have acquired from contaminated food or from other people.

Normally, we do not need to worry about resistant commensal bacteria in our bodies—they are just as harmless as their drug sensitive relatives. However, commensal bacteria can cause nasty and life-threatening diseases if they end up in places where they do not belong, e.g. in the blood stream or in the lung. Many infections acquired during hospital stays are caused this way, e.g., *Escherichia coli* that are normally found in the gut or other commensal bacteria can get into the blood stream through catheters, causing sepsis. Treatment of these diseases can be extremely complicated when the disease-causing bacteria are antibiotic resistant. Methicillin-resistant *Staphylococcus aureus*, or MRSA, has caused problems for just this reason: it is a commensal bacterium that can turn pathogenic and cause bloodstream infections, pneumonia, or infections at surgical sites. MRSA are often resistant to multiple antibiotics of various classes, making infections hard to treat.

Antibiotic resistance without antibiotics:
A soldier in World War I and an Amazonian tribe

In 1915, a young British soldier, Ernest Cable, was hospitalised in a French hospital for dysentery. Dysentery is a diarrhoeal disease caused by bacteria of the *Shigella* species. It cost many their lives during World War I and continues to do so in developing countries. Unfortunately, Ernest Cable did not survive. However, the bacteriologist of the hospital isolated the bacteria, and they were deposited in the UK National Collection of Type cultures. About 40 years later (in the 1950's), the bacteria were tested for antibiotic resistance. It turned out that they were resistant to penicillin and erythromycin. Almost 100 years after Ernest Cable's death, researchers sequenced the genome of the bacteria that had killed the soldier, and they found the genes conferring resistance to these two antibiotics. Now look at the dates: penicillin was first discovered in 1928 and only widely used much later, and yet, some bacteria were already resistant to it in 1915!

Two years ago, researchers investigated the commensal bacteria of a remote Amazonian tribe, the Yamomami, for antibiotic resistance. As far as it is known, the Yamomami have never had contact with Western people before, and have never taken antibiotics. And yet, their bacteria carry antibiotic resistance genes!

These findings are perhaps less surprising when we consider where antibiotics come from. Most antibiotics are derived from natural compounds (remember pyocynase and penicillin from the introduction). Only very few are fully synthetic. That means bacteria have been confronted with these compounds long before we started using them as medicines, leading to the evolution of bacterial defence mechanisms.

Resistance in the commensals can turn problematic for a second reason. If the resistance genes are located on plasmids, they might be transferred from commensals to pathogens. In fact, researchers have discovered that this has already happened. *E. coli* and *Klebsiella* passed on their resistance genes to *Salmonella* within the human gut during treatment. In that case, the commensals are not themselves culprits, but do serve as reservoirs of resistance genes.

Bacteria are complex organisms with great adaptive potential, and they are many. Whenever we use antibiotics, we exert a selection pressure on bacteria that could lead to the evolution and spread of resistance. Even if we use high doses or combinations of drugs, a risk of resistance remains. This is evolution happening in our bodies, in the bodies of our farm animals, in our waste water. And it is rapid. Every time a new antibiotic is released onto the market, it usually only takes a few years before the first resistant cases are detected. The development of new antibiotics cannot keep up with the speed of evolution. So what can we do? The best way to slow down the evolution of resistance is to reduce the selection pressure, i.e. to use fewer antibiotics, and when we do, to use them intelligently. But what does "intelligent use" entail? Well, for that we need to understand more about evolution...

Important terms

pathogenic bacteria: bacteria that cause disease
broad-spectrum antibiotic: antibiotic that is effective against many different bacterial species
plasmid: DNA element that is separate from the chromosome; antibiotic resistance genes are often located on plasmids. Some plasmids can "infect" other cells on cell-to-cell contact.
commensal bacteria: bacteria that we carry in and on our bodies. Harmless under normal circumstances, they can also turn into pathogens

Useful resources

- **World Health Organization (WHO), Fact sheet on antibiotic resistance:** `www.who.int/mediacentre/factsheets/antibiotic-resistance/en/`
- **Information about antibiotic resistance from the Centers for Disease Control and Prevention (CDC):** `www.cdc.gov/drugresistance`
- **Timeline showing the introduction of many antibiotics and the detection of the first resistant case:** `www.cdc.gov/drugresistance/pdf/5-2013-508.pdf`
- **Alexander Fleming's Nobel Lecture:** `www.nobelprize.org/nobel_prizes/medicine/laureates/1945/fleming-lecture.pdf`
- **"Antibiotic resistant bacteria at the meat counter":** `evolution.berkeley.edu/evolibrary/news/130501_superbugs`
- **Watch antibiotic resistance evolve in the lab:** `www.youtube.com/watch?v=plVk4NVIUh8`

Instructions for the competition

Questions:

Answer the questions from the Questions section. A question may have multiple correct answers. You receive the maximum number of points as indicated for each question if you identify all of the (and only the) correct answers. Send your answers in a format that gives the question number and the correct answer(s), e.g. "Q1: A, B, C; Q2: B, C, D;..."

The project:

Read the instructions in the Project section. You can get up to 20 points for this part. After completing the project, send us:

- The hypothesis that you formulated prior to the simulation.
- The completed Tables 2-4 and the graphs that you produced based on the tables.
- Your analysis of the simulations, guided by the questions provided in the Project section.

Also send us explanations for your answers in the project section, so we can give you partial points if you get something almost—but not quite—right!

Send the write-up of the project together with the answers to the Questions section, preferably as a single PDF, **by midnight on January 18, 2018** to **EvoBioSeminar@gmail.com**, or via post to **STEB, IST Austria, Am Campus 1, 3400 Klosterneuburg, Austria.**