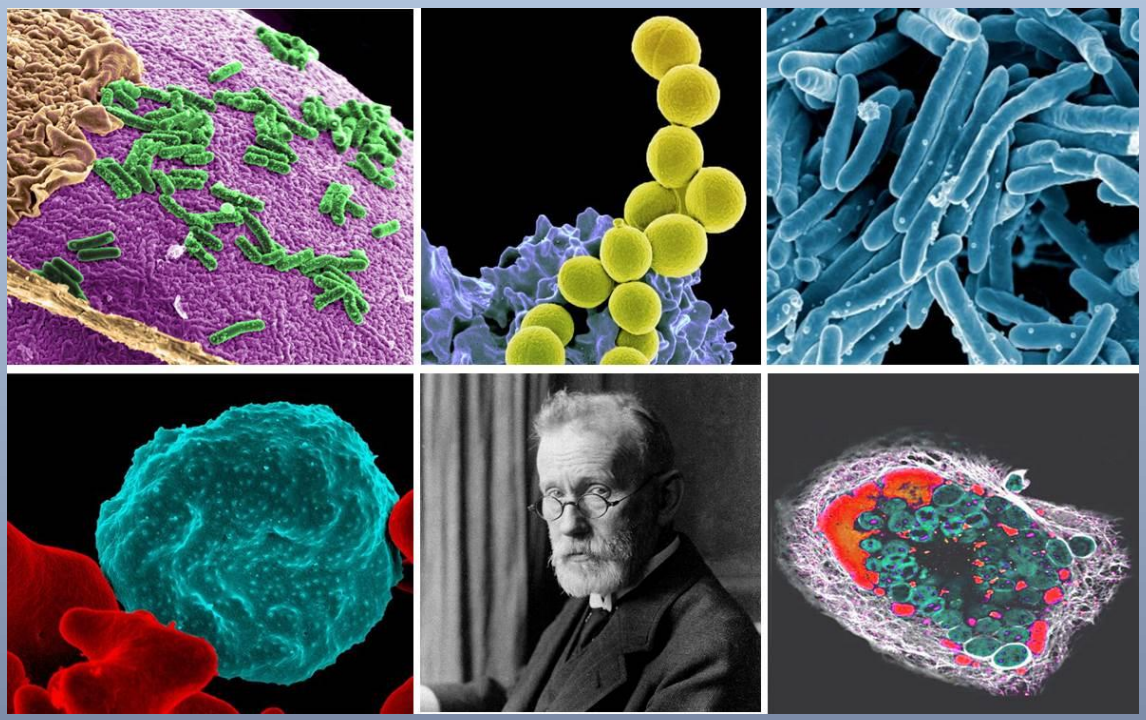
University of Glasgow



Microessays 2016

Science Communication in Action

A collection of short articles about microbiology written for the general public by Microbiology, Virology and Parasitology students from the University of Glasgow.

Front cover image details

Clockwise from top left: colourised electron micrograph of human intestinal cells cultured with gut bacteria [courtesy of Pacific North West National Laboratory]; colourised electron micrograph of methicillin-resistant *Staphylococcus aureus* bacteria (MRSA; yellow) being ingested by a neutrophil (purplish blue) [credit: NIAID]; scanning electron micrograph of *Mycobacterium tuberculosis* bacteria [credit: NIAID]; Rinderpest virus-infected cells [credit: AJC1]; Paul Ehrlich (1854-1915) [Wellcome Images: M0013322]; colourised electron micrograph of red blood cell infected with malaria parasites (blue). The small bumps on the infected cell show how the parasite remodels its host cell. Uninfected cells (red) have smoother surfaces [credit: NIAID/RML].

Contents

Foreword

Dr Tansy Hammarton, Senior Lecturer, University of Glasgow

It is becoming increasingly important for scientists to be able to communicate complex scientific topics clearly, simply and in an engaging way to a general audience. Not only do taxpayers fund a considerable amount of scientific research, earning them the right to know and understand what has been achieved with their taxes, but the importance of increasing scientific literacy amongst the general public cannot be underestimated. A greater understanding of the scientific data and processes underlying key issues allows more informed choices and debate, and can lead to improvements in health and wellbeing, raised aspirations, and valuable inputs into the research process by members of the public.

In 2015/16, to help them develop their science communication skills, I set our final year Microbiology, Parasitology and Virology students from the University of Glasgow the task of writing an accessible and engaging science article on a subject of their choice that fitted within one of the following four broad topics:

- History of infection biology
- The threat of emerging anti-microbial resistance
- Eradication of an infectious disease
- Exploiting endosymbionts to control infectious disease

The students wrote an 800 word draft, which was reviewed by myself as their course tutor and their fellow students, and then edited and extended their articles to 1200-1500 words before submitting them for marking. The grades students received for their articles contributed to their overall final degree grades. The students really engaged with this assignment, with many producing highly entertaining and informative articles. They were encouraged to enter their articles in the Biochemical Society 2016 Science Communication Competition, a competition open to undergraduate and postgraduate students from across the UK. Of the students who entered their articles in the competition, the articles of four students – Lauren Carruthers, Heidi Forsyth, Carlos Gamio and Jennifer Hallam - reached the final shortlist, a tremendous achievement. Here are their articles, along with a selection of the best of the rest. Enjoy!

History of infection biology

Of Rabbits and Men: the Tale of Paul Ehrlich

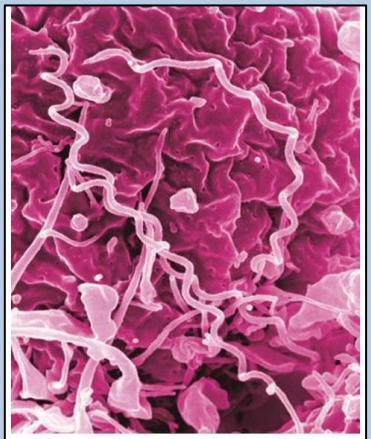
Carlos Gamio

Shortlisted for the Biochemical Society Science Communication Competition 2016

In our modern world of chemotherapy, antibiotics and antivirals, it might come as a surprise to find that the origin of all these treatments can be traced back to rabbits - the cute and fluffy kind. To understand why, we need to go all the way back to 1882 Berlin. A talented, if aimless, young German doctor, Paul Ehrlich, had just met the great microbiologist Robert Koch. Koch was giving a lecture in which he identified the pathogen responsible for tuberculosis. Ehrlich was instantly fascinated by Koch and microbiology. Unknown to himself, he had just taken the first step on a path that would help change the way disease is tackled forever[1].

Paul Ehrlich in his office in Frankfurt, circa 1900 [credit: Waldemar Titzenhaler].

The late 1800's were a time of dynamic change in the sciences. Charles Darwin had proposed his Theory of Natural Selection and Thomas Edison had given us the light bulb. Amongst the many fashionable topics of the time, some biologists were fascinated by dyes, specifically the staining of living tissue. Spending all day bent over a microscope looking at the pretty colours might not seem like worthwhile science by modern standards, but these dyes had interesting properties. Dyes displayed a high level of specificity; they would only stain certain structures and pass through others. Ehrlich noticed this and soon started to think of applications for these properties.



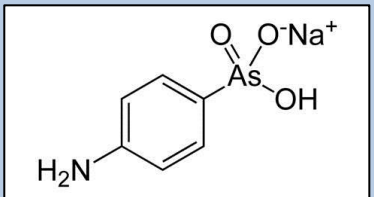
Treponema pallidum, the bacteria that causes syphilis [credit: NIAID, NIH].

These were times when catching a chill could kill. Many well-known individuals of the time were killed in their prime due to infectious disease. Emily Brontë died from tuberculosis[2], René Descartes from pneumonia[3] and Pyotr Tchaikovsky died from cholera[4]. However, one of the most notorious and stigmatising diseases out there by far was syphilis. Syphilis is a bacterial sexually transmitted disease (STD) and was known at the time as the great pox. As the name suggests, the disease was characterised by pus-filled pustules. Untreated, the disease could lead to heart conditions, deformities and death[5]. Dr. Ehrlich believed

that the selectivity observed with dyes could be harnessed to attack the bacterial organism responsible for syphilis, without damaging human tissue. Being an avid hunter, he likened it to having a "magic bullet" to kill the bacteria.

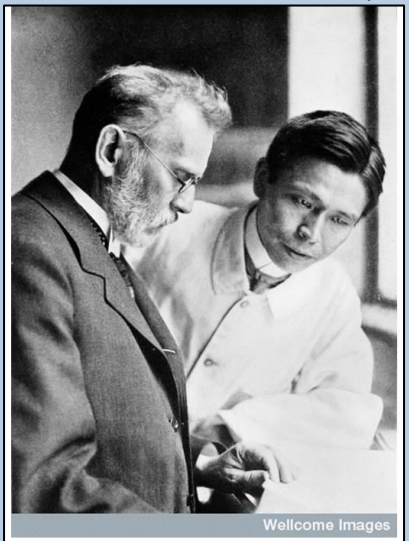
Magic bullets made from dyes do not sound like the most promising start for serious science, but this was a different age. Walking into a pharmacy in the 1800's would be a very interesting experience. Is your child teething and won't stop crying? No problem! The hefty dose of morphine in our all purpose soothing syrup should do the trick. Not to mention the use of mercury for STDs, bloodletting with leeches and the prescription of heroin for a cough[6]. To say that these had mixed results is an understatement, although by all accounts, heroin was excellent at dealing with a cough. Common side effects could include increased tolerance, addiction[7] and sudden loss of self-respect.

So what about the rabbits? You were promised fluffy and cute and the story delivers. Unfortunately for the rabbits, it is in a thoroughly unpleasant fashion. By 1899, Erhlich had been appointed director of the Royal Institute of Experimental Therapy in Frankfurt. In this role, he had free reign to explore his idea of "magic bullets" to attack disease. His work was initially unfocused and he worked on parasitic infections as well as bacterial infections. It was his work with sleeping sickness, a parasitic disease spread by the tsetse fly[8], which helped him identify an anti-parasitic arsenic compound called Atoxyl. This discovery encouraged Ehrlich in his search for a compound to treat and cure syphilis. The rabbits had the unfortunate job of being his test subjects[1].



Chemical structure of atoxyl.

Rabbits were known to be susceptible to syphilis and so made excellent test animals. However, to the rabbits' added misery, Ehrlich had no idea which arsenic compound would be effective as a magic bullet. Arsenic, as we all know, is not something to be adding to your tea. Often referred to as the inheritor's powder, arsenic trioxide was *the* poison of choice in the 19th century[9]. How could such a dangerous substance ever be thought of as a cure? The answer is that not all arsenic compounds are poisonous to mammals. Ehrlich knew this, and set out to find one that would attack the infecting bacteria but not the rabbits.

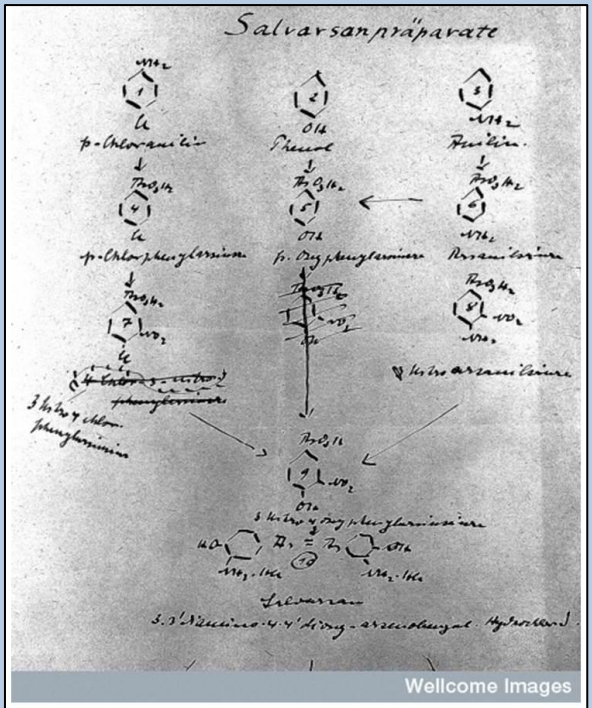


Paul Ehrlich and Sahachiro Hata [credit: Wellcome Images M0013258].

His approach largely relied on meticulous testing, strict organisation and a good helping of wishful thinking. Not knowing which arsenic-based dye would do the trick, he and his Japanese assistant, Dr. Sahachiro Hata, had to test them all. Things were pretty bleak for the bunnies. If they were *lucky* they would have survived the poisoning, only to still be suffering from syphilis. The unlucky ones would be killed by the compounds used, all in the name of science.

In 1908 Ehrlich was awarded a Nobel Prize for medicine and physiology. He had built a reputation as an expert in immunology and was well respected in his

field. However the magic bullet still eluded him[1]. By 1909, the two microbiologists had gone through 605 different variations of arsenic molecules, testing them all on the infected rabbits. Compound 606, arsphenamine, finally displayed the properties the two scientists (and hundreds of rabbits) had been hoping to find. Ehrlich and Hata had stumbled upon a chemical that cleared the syphilis infection, but did not kill the rabbit. It would later be renamed as Salvarsan and be marketed as the first targeted treatment for syphilis. In later years, a new more soluble arsenic compound was also discovered by Ehrlich and Hata. Compound 914 was slightly less effective than Salvarsan, but it was easier to administer and was marketed as Neosalvarsan[10].



Ehrlich's writing and chemical formulae for the preparation of Salvarsan [credit: Wellcome Images L0012361].

Unfortunately the establishment, as is so frequently the case, was slow to realise the importance of Ehrlich's work. Ehrlich being Jewish, this reluctance was in no small measure due to anti-Semitic feeling within some sections of society. Additionally, many people considered syphilis a just punishment for those with a less than wholesome lifestyle. It was believed that the availability of a cure would lead to generalised debauchery and loose morals. Nonetheless, the step had been taken and both Salvarsan and Neosalvarsan would go on to help millions of people[11]. However, more significant was the attitude change brought about by Ehrlich and his work. He inspired many future researchers, including Alexander Fleming, who discovered penicillin. From this point on, science would strive to find more "magic bullets" and attempt to directly target the cause of disease. His meticulous approach towards drug discovery would also change the way new medicines were tested. Medicine took its first steps out of the dark ages thanks to Ehrlich and his rabbits. The age of chemotherapy had arrived.

References

1. The Nobel Foundation (1967). Paul Erhlich – Biographical. *Nobel Lectures Physiology/Medicine 1901 - 1921* [online]. Available at: https:// www.nobelprize.org/nobel_prizes/medicine/laureates/1908/ehrlich-bio.html [last accessed 12/12/2016].
2. Tompkins, J.M.S. (2016). Emily Bronte, *Encyclopædia Britannica Online*. Available at: http://www.britannica.com/biography/Emily-Bronte [last accessed 12/12/2016].
3. Smith, K. (2014). Descartes' Life and Works, *The Stanford Encyclopedia of Philosophy (Winter 2014 Edition)*, [online]. Available at: https://plato.stanford.edu/archives/win2014/entries/descartes-works/ [last accessed 12/12/2016].
4. Poznansky, A. (2016). Pyotr Ilyich Tchaikovsky. *Encyclopædia Britannica Online* Available at: http://www.britannica.com/biography/Pyotr-Ilyich-Tchaikovsky [last accessed 12/12/2016].

5. National Health Service (2016). *Syphilis.* Available at: http://www.nhs.uk/conditions/syphilis/Pages/Introduction.aspx [last accessed 12/12/2016].
6. Porter, R., (1999). The greatest benefit to mankind: a medical history of humanity. W.W. Norton, New York, USA.
7. National Institute on Drug Abuse (2014). DrugFacts – Heroin. Available at: https://www.drugabuse.gov/publications/drugfacts/heroin [last accessed 12/12/2016].
8. Headrick, D.R. (2014). Sleeping sickness epidemics and colonial responses in East and Central Africa, 1900–1940. *PLOS Neglected Tropical Diseases,* 8: 2-8.
9. Hempel, S. (2014). Arsenic – The Near Perfect Murder Weapon. *Huffington Post* [online]. Available at: http://www.huffingtonpost.com/sandra-hempel-/arsenic-the-nearperfect-m_b_4398140.html [last accessed 12/12/2016].
10. Chemical Heritage Foundation (2015). Paul Erhlich *Online Resource*. Available at: https://www.chemheritage.org/historical-profile/paul-ehrlich [last accessed 12/12/2016].
11. Lehrer, S., (2006). *Explorers of the Body: Dramatic Breakthroughs in Medicine from Ancient Times to Modern Science*, pp. 285-294. Doubleday, Lincoln.

On the Events That Made Malaria Control Possible

Magdalena Kujawska

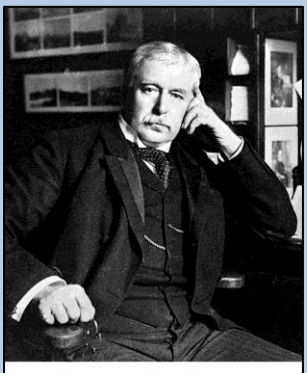
"…With tears and toiling breath
I find thy cunning seeds,
O million-murdering Death."[1]

— wrote Sir Ronald Ross about malaria, just days after making a historic breakthrough in our understanding of the transmission of this deadly and debilitating disease. On the August 20th 1897, the day since known as "Mosquito Day"[2], he discovered developmental stages of parasites causing human malaria in mosquitoes belonging to the genus *Anopheles* – an event which marked a milestone in our knowledge of the disease.

Sir Ronald Ross
(1857-1932)

As you are reading this, a child has died from malaria somewhere in the world. Each year malaria parasites infect more than 200 million people globally, and the disease takes more than 400 000 lives[3]. The overwhelming majority of deaths occur in African children under the age of five. Tiny, single-celled parasites of the genus *Plasmodium* are the culprits of disease, hiding and multiplying inside human red blood cells until the cells explode. We have known for more than a century that mosquitoes pass *Plasmodium* parasites to humans during the blood meal, but the road to the discovery of malaria transmission was neither quick nor easy.

Despite circumstantial evidence accumulated over centuries that mosquitoes might somehow be involved in spreading malaria, the 19th century malariologists could not explain how the disease was transmitted from one individual to another[2]. The theories proposing poisonous vapour (miasma) or vegetative ferments from the marshes as causative agents of the disease were widely accepted as recently as the very late nineteenth century[1].



Sir Patrick Manson
(1844-1922)

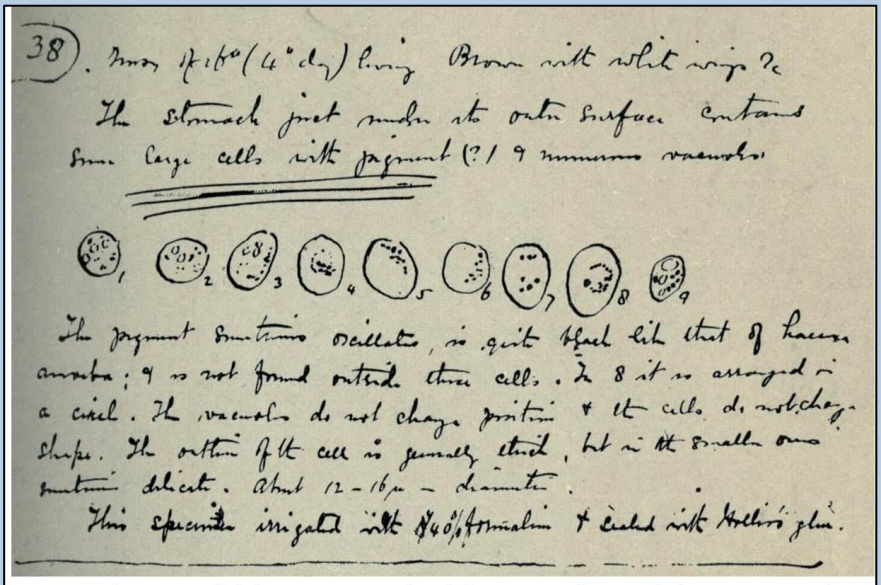
Between 1880 and 1897, a number of scientists had slowly become convinced that mosquitoes were connected with the transmission of malaria. Among them was Alphonse Laveran (who in 1880 had first identified *Plasmodium* parasites in the blood of a malarious French soldier), Patrick Manson (who in 1877 had observed that mosquitoes transmit worms

responsible for a tropical disease called lymphatic filariasis), and several Italian malariologists[2]. At that time, it was considered likely that humans acquired infection either by drinking water from sources containing infected mosquitoes or by inhaling dust from dried ponds in which infected insects had died[2].

During that period, Sir Ronald Ross was serving as a surgeon in the Indian Medical Service. He became interested in malaria in 1982, but after numerous failed attempts to find *Plasmodium* in the blood of malarious patients he started doubting the very existence of the parasite[4]. It was only after meeting with Patrick Manson during a period of home leave in 1894 that he acknowledged the presence of malaria parasites in the human bloodstream. Manson, who studied malaria patients in London, had observed that motile forms of the parasite appeared when blood collected from those patients cooled, and suggested that further parasite development occurred in another host, probably a mosquito[2].

On his return to India in 1895, Ross dissected several thousand mosquitoes experimentally fed on malarious patients from endemic areas without any success. All of those mosquitoes were either grey or brindled, belonging to the genera known as *Culex* and *Aedes*[2]. Eventually, two years and four months later, in August 1897 he made his discovery after his assistants, whom he affectionately called his "mosquito men", had caught a different type of mosquito – bigger than the others, brown and with dappled wings – a member of the genus *Anopheles*[5].

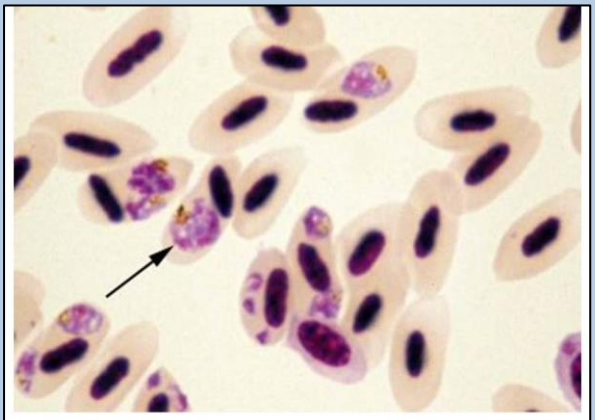
On August 16th, this new type of mosquito was fed on a malarious volunteer named Husein Khan, whose contribution to the research was compensated by a payment of 1 Indian anna per mosquito bite, an amount equivalent to 6 British pence[5]. Four days later, on August 20th, Ross found unusual pigmented cells in the stomach of one of the insects. This discovery was followed by an observation that the cells rapidly grew in the mosquito tissue until they ruptured and released rod-like structures, which then invaded the mosquito's salivary glands[2].


38. Anop. ♀ 16d (4th day) living Brown with white wings &c
The stomach just under its outer surface contains some large cells with pigment (?) & numerous vacuoles
The pigment sometimes oscillates, is quite black like that of haemamoeba; & is not found outside these cells. In 8 it is arranged in a circle. The vacuoles do not change position & the cells do not change shape. The outline of the cell is generally circular, but in the smaller ones sometimes delicate. About 12–16μ – diameter.
This specimen injected with 4% formalin & sealed with Kollin's glue.

Excerpt from Ross's laboratory notebook (August 20th 1897) documenting the discovery of large pigmented cells in the mosquito stomach [Credit: London School of Hygiene and Tropical Medicine Archive].

Ross came extremely close to proving that *Anopheles* mosquitoes could transmit human malaria but unfortunately he was interrupted form completing his studies. At

this crucial stage he was ordered to Calcutta, an area with very few human malaria cases[4]. At Manson's suggestion he focused his research on malaria parasites of birds, *Proteosoma relictum,* now known as *Plasmodium relictum*, and in July 1898 he discovered that these parasites were transmitted by grey *Culex* mosquitoes[2]. He proved that during the blood meal mosquitoes took up male and female cells from infected birds and that the cells fertilized and became pigmented in the mosquito stomach. He also observed the formation of the rod-like structures inside pigmented cells that then moved to the mosquito's salivary glands and were injected into a bird when the mosquito took its meal[2].



Plasmodium relictum parasites (arrow) in a canary blood smear (note that blood cells contain a nucleus, stained dark purple) [Credit: US Geological Survey].

Ross suspected correctly that human malaria was spread in the same way and proposed that one single experiment could test that hypothesis. However, his military duties once again interfered with his research. He was ordered to help with an epidemic of plague that was then raging in India and was prohibited from continuing his work on malaria[4]. In the meantime, Italian malariologists Bignami and Grassi produced the proof of the mosquito's involvement in the spreading of the disease. In their experiment they successfully transmitted malaria to uninfected individuals via the bite of local *Anopheles claviger* mosquitoes previously fed on infected patients[2]. Ross afterwards angrily accused the Italians of stealing his work and refused to acknowledge the independence of their research[4].

Following the discovery of the role of Anopheles in the transmission of malaria, the scientific efforts of malariologists focused on devising the means to control the disease. In a classical experiment, Grassi sent over a hundred volunteers protected from mosquito bites to one of the malarious areas in Italy, and found that only five of them acquired the infection compared to four hundred unprotected volunteers who all contracted malaria[2]. Over the next decades, methods to minimise human exposure to mosquitoes had become crucial in our fight against malaria.

One of the most ancient inventions – the mosquito net – has become essential in limiting contact with infected insects. Originally used as a simple barrier, it was first impregnated with an insecticide during the Second World War and became a useful tool in reducing mosquito numbers[6]. Today, insecticide-treated nets are distributed on a mass scale across countries suffering from malaria as part of a global programme of malaria control. In 2014, 189 million nets were delivered to households in countries of

Ceiling-hung mosquito net [Credit: Tjeerd Wiersma].

sub-Saharan Africa[3]. In addition, to limit mosquito access to houses wall openings are blocked and the walls are sprayed with insecticides.

Furthermore, several means to destroy mosquito habitats have been introduced in an attempt to prevent malaria transmission. Historically, pouring oil into the pools of standing water was used as a control method[6]. Nowadays, more measures of control are available. Methods such as draining the mosquito habitats, the use of chemical insecticides and bacterial toxins or the use of parasitic worms or fish as predators of mosquito larval stages have been implemented in 48 countries affected with malaria[3].

Mosquito larvae in standing water [Credit: James Gathany/CDC].

The World Health Organisation has identified mosquito control as the main measure to ensure to protection against malaria[7]. In the period between 2000 and 2015, the number of new malaria cases in the world decreased by 37% and the number of deaths from malaria fell by 60%[7]. Over the last decades we have made an important progress in our fight against this ancient disease. We should not forget that this progress was originated by Ronald Ross's dedication to solving the puzzle of malaria transmission.

References

1. Cook, G. (2007). Ronald Ross (1857-1932): the role of the Italian malariologists, and scientific verification of mosquito transmission of malaria. In: *Tropical Medicine: An Illustrated History of The Pioneers*, pp 81-102. Academic Press, Elsevier.

2. Cox, F. E. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites & Vectors*, 3:5.

3. World Health Organisation (2015). World Malaria Report 2015. Available from: http://www.who.int/malaria/publications/world-malaria-report-2015/en/ [Last accessed: 16/12/16]

4. Magill, F. N. (1999). The 20th Century O-Z: Dictionary of World Biography. Routledge, London & New York.

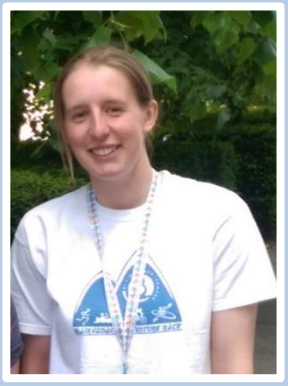
5. Ross, R. (1923). Memoirs, with a full account of the great malaria problem and its solution. London J. Murray.

6. Busvine, J. R. (1980). Insects and Hygiene: The biology and control of insect pests of medical and domestic importance. 3rd edition. Chapman & Hall.

7. World Health Organisation (2016). Malaria fact sheet. [Available from: http://www.who.int/mediacentre/factsheets/fs094/en/ [last accessed: 16/12/16].

The threat of emerging anti-microbial resistance

The Antibiotic Resistance are Fighting Bac – teria

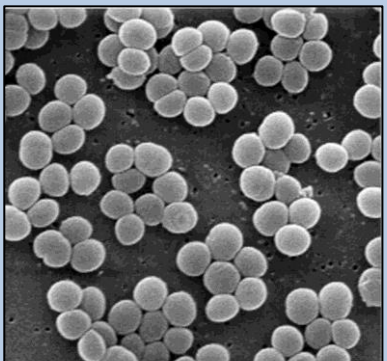


Lauren Carruthers

Shortlisted for the Biochemical Society Science Communication Competition 2016

In the dead of night, Ambrose sneaked out of the hospital and into a dark alleyway to join The Antibiotic Resistance – a group of bacteria determined to fight back against doctors and save their species from eradication. It was there that he became a member of the MRSA (methicillin resistant* Staphylococcus aureus*): an elite team equipped to survive extreme conditions and cause deadly diseases.

Antibiotics, the collective term for the medications used to treat bacterial infections, have saved millions of people, improved quality of life and progressed livestock farming. A world without antibiotics would be bleak. Prior to antibiotic discovery the majority of bacterial meningitis cases were fatal[1]; therefore, more people would die if antibiotics were ineffective. Without antibiotics, surgical procedures would not have advanced[2]. Their failure now would lead to a backwards step in almost all aspects of medicine. Patients would risk succumbing to infection after surgery, breaking bones or puncturing the skin. People with weakened immune systems (our bodies' own defence mechanism against infection) due to old age, cancer or viral infection would increasingly perish. This is scary before we even mention food security problems if animals became diseased. Unfortunately these threats could become a reality as troops of bacterial species, including *Staphylococcus aureus*, are withstanding antibiotic therapy raids.

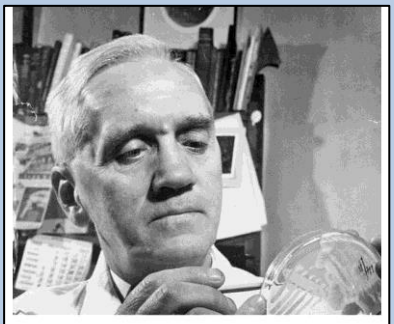


Scanning electron micrograph of *Staphylococcus aureus*

Staphylococcus aureus is a bacterium which can cause a range of infections. It can live on the skin with no negative impact, trigger unpleasant rashes and boils, or inflict a serious infection such as meningitis and blood poisoning with lethal consequences. Fortunately deaths associated with bloodstream infections have reduced up to 63% after antibiotic introduction[3]. Around 30% of us carry *Staphylococcus aureus*[4], usually on our skin and in our nasal cavities. Whilst very few people exposed to the pathogen develop severe symptoms, it does mean that Staphylococci are disseminated in the population ready to ambush their next victims. A wide spectrum of disease has also been

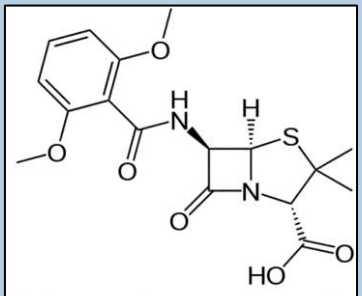
observed in domestic and wild animals[5] which have the potential to transmit the bacterium to humans. Humans can also pass it on to animals[5]. Therapy used to be effective but currently treatments are failing. Unfortunately this means suffering and death rates resulting from this bacterium are rising. This is a major problem. But why are these antibiotics now unsuccessful?

In 1928, Alexander Fleming discovered a mould that produced a natural antibiotic that prevented growth of *Staphylococcus aureus* which he called penicillin. Eleven years later saw the start of World War 2 and massive effort to produce large quantities of this antibiotic to cure wounded soldiers subsequently infected with a range of bacteria. As a result, many men were saved and penicillin became widely used. ***However, as one war ended, another was brewing – an army of bacteria had gathered together to fight against penicillin. They established The Antibiotic Resistance and found an effective weapon, an enzyme called penicillinase, which, like a pair of scissors, is able to cut penicillin, rendering it inactive.*** Records show that penicillinase was first known to be used by *Staphylococcus aureus* in 1944[5]; since, it has been passed on to ~95% of their species globally[5]. So, the battle was back on! Only this time, it was microbial warfare.



Alexander Fleming at work in his lab at St Mary's Hospital, London in 1944 [credit: Collections of the Imperial War Museums D17802].

Methicillin, an antibiotic which has the same pharmacophore (the active part of a drug) as penicillin, was then created. Imagine a Christmas tree - this is the key component or the 'active part', if we think of it like a drug. We can add lots of different things to it: tinsel, baubles, stars, angels, beads, in lots of different colours: blue, green, red…… These different components can be added or taken away to create lots of different effects, but underneath it all, it is still a tree. The same principle can be used with pharmacophores – lots of different chemical parts can be added or removed. What is so special about methicillin? It has been designed to withstand penicillinase and kill penicillin-resistant bacteria.



Chemical structure of methicillin.

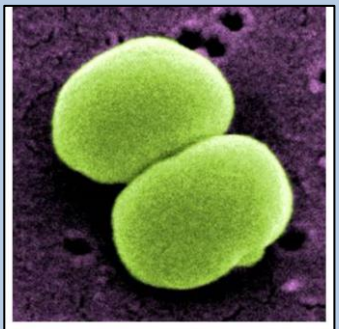
The bacteria were not about to surrender yet: an elite group fitter than the rest of the army, like the SAS, formed within The Antibiotic Resistance. They called themselves the MRSA. They discovered a special* mecA *gene: a code which when deciphered provided them with a set of instructions enabling them to disarm the drug.

So, how did Ambrose become a member? Well, bacteria have evolved several mechanisms to transfer DNA made up of lots of gene codes between each other, and like humans exchanging ideas, the most useful ones are kept circulating. A

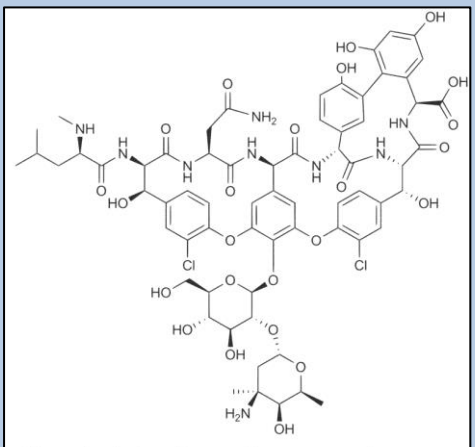
member of the MRSA was simply able to pass Ambrose a *mec*A gene, armouring him against methicillin.

But where did the *mec*A gene come from? No-one is really sure[6]. Perhaps the code was naturally accessible. Nutrients are limited in the environment. Bacteria, moulds and other microbes have to compete for these nutrients. Some deploy antibiotics to kill off their rivals; however, they must tolerate their own venom, and one way of doing this is carrying the information to decode it[7]. Thus, for every natural antibiotic produced, a set of guidelines to defuse it are possibly present in the environment. It only takes one bacterial spy to extract and pass on this information for bacteria to become resilient. This data distribution is scientifically known as horizontal gene transfer. There are several forms like phone, e-mail and post, and it is the method used to send messages, such as the antibiotic deactivation cyphers, between, or to other, microbial species.

Resistance can arise through another natural process: mutation. Mutations are basically errors made in the codes when bacteria copy them. Most translate the code to gobbledygook and the bacteria die. Sometimes, they are extremely helpful. Whilst antibiotics kill most bacteria, they startlingly also select for those that are the most dangerous[2] – those with mistakes that spell antibiotic survival. The lucky few, tough enough to endure antibiotic exposure, thrive because they suddenly have access to plenty of uncontested nutrients! Bacteria have a rapid replication time and multiply exponentially (1, 2, 4, 8, 16, 32, 64, 128…), so can soon generate a sizeable armed force, although, with maintained efficient antibiotic use, even they will die off before mass deployment. However, if an antibiotic course is stopped early, or is ineffective, these bacteria can dominate with dangerous consequences, so it is vital you take your antibiotics as prescribed! Furthermore, through mutations, bacteria can perfect horizontally-transferred genes to enhance their knowledge about antibiotic deactivation.



False coloured scanning electron micrograph of dividing *Staphylococcus.*



Chemical structure of vancomycin.

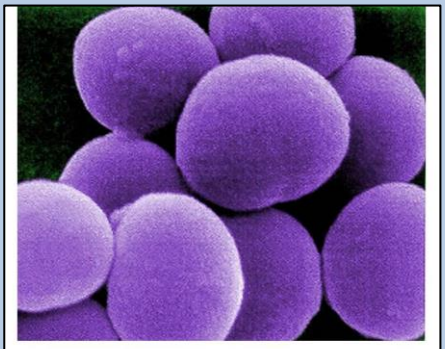
Whether by theft or chance, the *mec*A gene was obtained. The Antibiotic Resistance were victorious once again. So we reloaded with the new antibiotic vancomycin. Unfortunately the VRSA (vancomycin resistance *Staphylococcus aureus*) retaliated. Alas, our ammunition is now running low; we have very few useful medications to treat bacterial infections in reserve. Scientists are presently in pursuit of new antibiotic compounds, but the assignment is lengthy. It takes many years to discover new medications and get them safety approved, so our defences

could be down for some time.

Negligence on our part has helped establish The Antibiotic Resistance. In a modernised world we've selfishly pumped chemicals out into the landscape. Basically, we've exposed bacteria to sub-lethal levels of poisons, giving them opportunity to concoct an antidote. Overuse and incorrect dosing of antibiotics present the same problem. These issues potentially increase the likelihood of bacteria being able to put up a decent battle to previously seen or unseen antibiotics in the future[8]. Carefree hygiene approaches also mean harmful bacteria inhabit our homes and reside on our bodies. To reduce the risk of antibiotic resistance, attitudes need to change. People have to realise antibiotics will not cure their virus infection and prevention, such as hand washing, is better than cure. This would buy us more time to find drugs.

Worryingly over the years, The Antibiotic Resistance have recruited numerous species including *Escherichia coli*, *Mycobacterium tuberculosis* and *Clostridium difficile*. They have also occupied many settings including hospitals, farms, schools and public transport systems on a global scale. Chances of contracting untreatable infections are undoubtedly getting BIGGER. The threat from The Antimicrobial Resistance is eminent. It endangers our lives and life as we know it.

Meanwhile, back in the hospital, Ambrose had multiplied to create the Ambrosia. They infiltrated the toilets, light switches, door handles and fruit bowls, then hitch-hiked from patient to patient. As a deluge of people began to vomit, groan and faint, staff prescribed methicillin. Only, it didn't work. Next they tried vancomycin, but the VRSA had backed up. Reserve medications were injected into the heat of the battle. Some patients improved. Others continued to decline. Nothing could be done. They died.



False coloured scanning electron micrograph of vancomycin-resistant *Staphylococcus aureus* [credit: CDC/PHIL/Janice Haney Carr].

This is the threat that awaits us as more bacteria resist antibiotics.

References

1. National Health Service (2014). Meningitis. Available from: http://www.nhs.uk/Conditions/meningitis/Pages/introduction.aspx [last accessed 15/12/2016].

2. Ventola, L. (2015). The antibiotic resistance crisis part 1: causes and threats. *Pharmacy and Therapeutics*, 40: 277–283.

3. Van Hal, S. B. *et al.* (2012). Predictor of mortality in *Staphylococcus aureus* bacteremia. *Clinical Microbiology Reviews*, 25: 362–386.

4. Centers for Disease Control and Prevention (2011). *Staphylococcus aureus* in healthcare settings. Available from: http://www.cdc.gov/HAI/organisms/staph.html [last accessed: 15/12/2016].
5. Grena, H. A. *et al.* (2015). Methicillin resistant *Staphylococcus aureus* (MRSA): a review. *Advances in Animal and Veterinary Sciences*, 3: 79–98.
6. Hiramatsu, K. *et al.* (2001). The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. *Trends in Microbiology*, 9: 486–493.
7. Benveniste, R. and Davies, J. (1973). Aminoglycoside antibiotic-inactivating enzymes in Actinomycetes similar to those present in clinical isolates of antibiotic-resistant bacteria. *Proceedings of the National Academy of Sciences,* 70: 2276–2280.
8. Berendonk, T. U. *et al.* (2015). Tackling antibiotic resistance: the environmental framework. *Perspectives*, 13: 310–317.

Bac-teria Future

Heidi Forsyth

Shortlisted for the Biochemical Society Science Communication Competition 2016

It's 1925. The time of flapper dresses, jazz and Charleston dancing, elegance and glamour.

And the time where STDs are collected like Al Capone does enemies (and victims alike), and typhoid fever is a common added extra to your drinking water[1].

Except it's not 1925. It's 2035.

And you're coughing and coughing and it won't stop. And you can't breathe, your skin a fiery blaze engulfing you in waves but, at the same time, you can't stop shivering. And there's nothing anyone can do.

"Give me an antibiotic!" you'd beg today. But that's the very problem: we've not got any left; none that work anyway. And our health care is just like the 1920s again, no glamour or sophistication, and no antibiotics.

~

Okay, so we're not there yet. The only thing in our hospitals and surgeries resembling the 1920s right now, is the slightly dated décor. And maybe a few of the waiting room magazines.

So sure, we're *hopefully* not going to be faced with 1920s health care. But that's the thing: we're only "hopefully". We need more time. Or else we will be sitting in hospital rooms, dying of infections that we shouldn't be, (probably still reading about The Kardashian's) twenty, thirty years from now[2]. But let's not worry too much, right? Because for now we still have our antibiotics, don't we?

Antibiotic capsules.

Antibiotics. Oh, as soon as you even say the word, you feel better! They were the gift to doctors everywhere – shout out to Al' Flemming for being a shockingly grotty lab worker! Penicillin, and the other antibiotics that followed, were like the best toys doctors got for Christmas; used and used to excess

until they struggled to keep up. Now the batteries are all falling out, held in with sticky tape, and needing a few hard hits to get them going.

Because antibiotic resistance happened. And, like our best toys, antibiotics are beginning to stop working.[3]

See, when bacteria replicate, they're slightly narcissistic in that they make their next generation exact copies of themselves. Which is fine, but sometimes when they make these copies, a mutation[4] happens. Think X-Men: they're still the same old *E. coli* or *Salmonella*, but better. So something goes a little differently in their genes (the little nuggets of DNA that make them how they are) and that in turn changes something about them. If that change is bad, then these mutants won't survive alongside the identical cool kids that are already there, and they'll not be around for very long. But if it is a good thing, then *they* become the cool kids that everyone wants to be like.

And antibiotic resistance *is* a good thing. For bacteria…..not so much for us. They got the power card there, the blue shell in Mario Kart that blasted us out of first place.

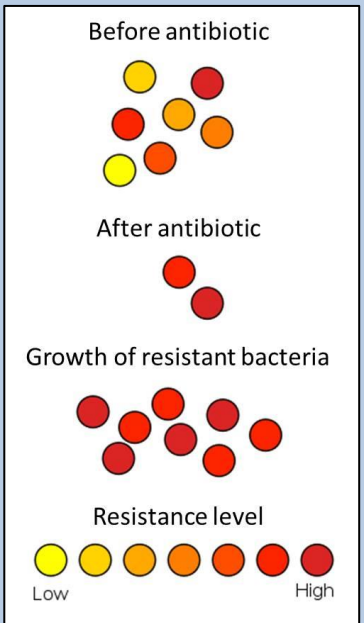
So the X-Men bacteria with their superpower of antibiotic resistance won't normally be much of a problem since there aren't that many of them, right? Compared to all the other bacteria there that don't have the mutation, they won't be able to take over from one little mutant?

They do if we help them. And we do. We actually help the bacteria to make us sick. - *Wait, what?*

Hands up who's ever not finished a course of antibiotics? Or decided you were a doctor/pharmacist (sans appropriate qualification) and pinched some of someone else's? Or brought back an old pack you had lying around? (Don't worry, even my hand is up).

When we do this, rather than getting rid of the bugs making us ill, we select for the ones that resist the antibiotic[4,5]. So we start with the antibiotics all ready to take on the infection, in and out, mission complete. Except we're only able to get to some of the bacteria that are there. The ones with the antibiotic resistance have their shields up and can just wait it out. And the antibiotics might as well be punching a brick wall to those bacteria who just shrug and keep on replicating, making more and more of their antibiotic resistant family.

And that family of antibiotic resistant bacteria can go on and infect other people. And then what do we treat them with? And everyone that they spread the infection to as



The development of antibiotic resistance. Circles represent bacteria and are colour-coded according to their antibiotic resistance.

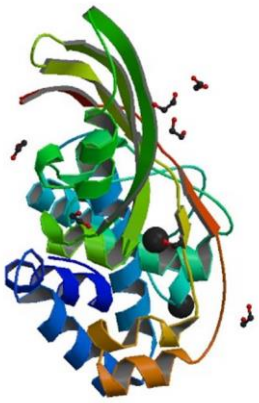
well? Another antibiotic, sure. But what happens if the bacteria evolve to resist that antibiotic too? And the next one? What happens when we run out of antibiotics to try?

And we will run out of antibiotics.

In the last 50 years, we have only managed to discover two antibiotics[6]. 2, deux, dos, TWO! And the thing with antibiotics is that we can group them based on how they work. Some like to target things on the outside of the bacteria, some can get right in there and start messing with their metabolism so they can't make any of the things they need to survive. But they target specific things. And with bacteria evolving to resist the antibiotic, this can mean the bacteria lose or hide that target. So even if we find more antibiotics they'll most likely only be able to work on or target something that they no longer have access to. And when the bouncer says you're not getting in, then you're probably not going to get in. And neither are your pals.

So we need time.

Because we *are* trying. Different ideas to keep us ahead of bacteria are under development by scientists all over the world, working tirelessly, desperately for us to have *something*. I could list a bunch here and still not have covered even a smidge of all the ideas being looked into. Bacteriocins: proteins that are made by bacteria that will specifically target, and importantly get rid of, another type of bacteria closely related to them, but not the same strain. This really specific targeting is also good for keeping our own good bacteria that live in us happily and harmlessly (known as our microbiome) in balance. Antibodies: another protein our body makes already to get rid of infection but pre-prepared and given to us as a drug to speed along the actual "getting-rid-of" part. Enzymes, proteins made by any living cell that can be used in a lot of different ways, like to stab holes in the bacteria[7-8] or to steal essential nutrients away from the bacteria before they can get them, making them starve[9]. We have options and many, many different bacterial assassins to consider. But they're all still getting ready, still being developed and made safe for us. We're still briefing our next generation of bacterial killers while bacteria keep getting more and more resistant. It's a game of cat and mouse and, right now, we're the mice.



Crystal structure of the bacteriocin 'Syringacin M' showing the ribbons and helices of the protein chain [credit: Grinter, Roszak, Cogdell, Milner and Walker. 4FZL.pdb].

There are a lot of ways to go, a lot of paths for us mice to run down. But we're probably best learning from our first mistake when we used and abused antibiotics. We have options and we should use them all when we can. Bacteria are unpredictable and we need to be too. The boxer, Floyd Mayweather Jr once said,

"He can hit harder and he can be stronger, but there's no fighter smarter than me." That's us: boxers stepping into a ring, sprinters on a track. Except this isn't a game; this is our lives. We don't get to try again or have a rematch. We need to be smarter than the bacteria, we need to be, not one step ahead, but three steps ahead. We have to keep on our toes and keep guessing the next move of the bacterial population so we can time our attacks well, and keep our defence up. The race is on, it has been on for years, and they're catching up on us. We can't let them. Because if we do, there's good chance that we'll be the ones knocked out for good.

It's 2016 and we still have antibiotics. *But for how long?*

References

1. MedicineNet.com (2015). Typhoid fever. Available from: http://www.medicinenet.com/typhoid_fever/article.htm [last accessed 12/12/2016].
2. Davies, S. *et al.* (2013). 'The drugs don't work. A global threat' Penguin books. Summary available from: http://www.bbc.co.uk/news/health-21178718 [last accessed 12/12/2016].
3. Wise, R. *et al*. (1998). Antimicrobial resistance is a major threat to public health. British Medical Journal, 317: 609-610.
4. Davies, J. and Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews,* 74: 417-433.
5. National Electronic Library of Infection (2016). Bugs and drugs on the web. Antimicrobial resistance. Available from: http://www.antibioticresistance.org.uk/ARFAQs.nsf/all/538166F256EC45B980257169004FFE8C?OpenDocument [last accessed 12/12/2016].
6. Coates, A. R. M. *et al.* (2011). Novel classes of antibiotics or more of the same? *British Journal of Pharmacology* 163: 184-194.
7. Borysowski, J. *et al.* (2006). Bacteriophage endolysins as a novel class of antibacterial agents. *Experimental Biology and Medicine*, 231: 366-377.
8. Staphefekt by Microes (2016). https://www.staphefekt.com/en/ [last accessed 12/12/2016].
9. Farnaud, S. and Evans R. W. (2003). Lactoferrin—a multifunctional protein with antimicrobial properties. *Molecular Immunology*, 40: 395-405.

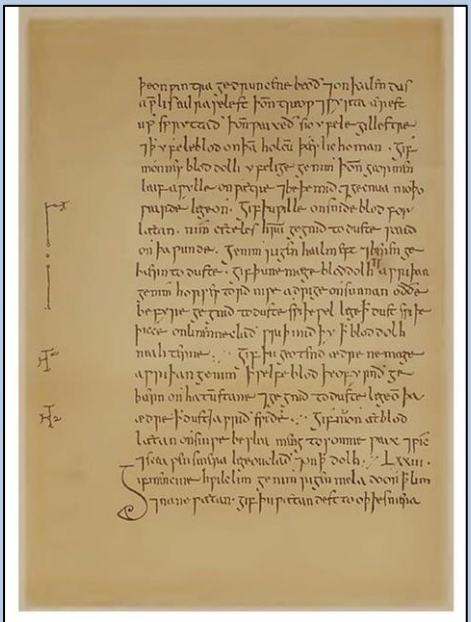
A Quest for Novel Antimicrobials

Jennifer Hallam

Shortlisted for the Biochemical Society Science Communication Competition 2016

It is cold and damp, the stench of manure and soil cling to the air. Under a thatched roof, a physician fumbles between rows of glass tubes containing various concocted remedies. He turns, waving frantically, holding a vile containing a peculiar coloured liquid. He pronounces that this will treat the young man's troubled "wen". Some days pass and the young man returns to the physician, proudly announcing his eye has healed and the ailment has gone.

Fast forward 1000 years. Men and women alike, line up along the sides of a battle ground. Wooden sword in one hand, shield in the other. The sun is beating down and birds sing in the nearby trees, the dull knocks echo into the forest, as sword meets sword. Over refreshments, after a hard battle, the actors discuss what life must have been like in Anglo-Saxon Britain, sparking curiosity amongst a few. In particular, a microbiologist wonders how infections from battle wounds were treated during this time, in the absence of medical and scientific knowledge.



A page from Bald's leechbook from the 10th Century, published in 1865 by Oswald Cockayne [British Library Royal MS 12 D XVII].

Collaboration with the University of Nottingham Humanities department reveals an ancient physician's book which could provide the answers she has been searching for. In amongst the thousands of historical manuscripts, *Bald's Leechbook* remains, a collection of ancient antimicrobial remedies recorded within. A remedy is identified that appears to treat a sty of the eye, a common microbial infection of the eyelash follicle caused by *Staphylococcus aureus* – which naturally lives on our skin. The old English language is difficult to understand. However, a translation uncovers that various *Allium* species including garlic and leek are essential ingredients, as well as some other irregular specimens including ox gall.

You might well be asking yourself why some ancient recipe would have a microbiologist so excited. The reason is simple; it could be the Holy Grail they've been desperately searching for.

Antimicrobial resistance is thriving. Over recent years, scientists have been at a tug of war with bacteria; with each new antimicrobial development, bacteria have fought back and evolved a new tactic for evasion. Scientists are particularly concerned with the growing bacterial resistance emerging against commonly used broad-spectrum antibiotics for the treatment of minor infections. Antibiotics which were once effective at eradicating infection have since been rendered completely ineffective against some bacterial strains. This is a worrying concept, as before the golden age of antibiotics, bacterial infections were responsible for high mortality rates in many countries. The phenomenon of resistance is a natural process which occurs in bacteria. Genetic information is transferred by means of replication or horizontal exchange between different bacterial strains. During these processes, the bacteria obtain new information that enables them to resist antibiotics. The overuse and misuse of many of these antibiotics has accelerated this process and as a result resistant strains have emerged sooner than predicted.

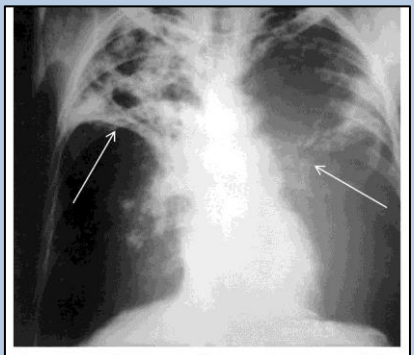
Infections caused by resistant bacterial strains are around 60% more deadly to humans than the non-resistant strains. In consequence of bacterial resistance, 25,000 deaths in Europe alone are estimated to occur annually by the World Health Organisation (WHO)[1]. MRSA (Methicillin-resistant *Staphylococcus aureus*) is one of the most significant examples. Over the past decade or so it has become renowned for its notorious ability to form resilient bio-films on invasive hospital instruments, resulting in increased cases of hospital acquired infections. This strain of *Staphylococcus aureus* is lethal, often leading to chronic wound infections and severe blood poisoning in individuals. MRSA was previously a treatable infection; however, the prevalence of blood poisoning cases increased dramatically throughout the late 1990s[2]. Furthermore, this bacterial species has developed multi-drug resistance. VRSA (Vancomycin-resistant *Staphylococcus aureus*) emerged completely resistant to available antibiotics during the beginning of the millennium and has presented doctors and scientists with an even greater challenge.

Scanning electron microscope image of *Mycobacterium tuberculosis* [credit: Alain Grillet/Sanofi Pasteur. Published under Creative Commons licence CC BY-NC-ND 2.0].

Silently creeping out from the dark cracks of the desolute slums, a second deadly bacterium is gathering momentum for a frontline attach on modern day society. *Mycobacterium tuberculosis* was responsible for widespread chronic lung infections throughout the working class population of the UK during the 19th century. The debilitating infection was accountable for nearly 4 million deaths from the 1850s to the early 1900s until the introduction of antibiotics and dissolution of slummed housing at the turn of the 20th century saw tuberculosis become a whisper of the past. However, in our absent mindedness, we neglected to maintain surveillance of these bacteria. *M. tuberculosis*, like many bacteria before, has sought out an opportunity to return and this time it has an

advancement – antibiotic resistance! We have been careless and as a result a disease once under control has crept back into our society. The recent emergence of resistance has become widespread. WHO estimated that there were 480,000 new cases of multi-drug resistant tuberculosis (MDR-TB) in 2015 alone[3]!

Occurrence of tuberculosis is particularly high in the UK, with figures released by WHO reporting that rates of infection in London are the highest in Western Europe. The incidence of disease is primarily concentrated in parts of the city which are highly deprived; lack of good sanitation and hygiene harbours these deadly infections. The recent trend of migration from countries where tuberculosis infection is prevalent is understood to be the leading cause of reintroduction of this infection to Western countries. What is more worrying is the prospect that there will be no useful antibiotics available to treat new cases of infection if this threat becomes more sinister. Already, extensively drug-resistant tuberculosis (XDR-TB) has been identified in more than 100 different countries[3]. Emergence of strains such as XDR-TB could lead to no treatments for tuberculosis and see mortality rates soar as a result.



Lung X-ray of patient with advanced TB. Arrows indicate TB infiltrate into airspaces of lung.

You're probably wondering how this all comes back to the Anglo Saxon recipe, right? Interestingly, Harrison's research group recreated this 'ancient soup' in a laboratory setting to investigate its antimicrobial properties[4]. To their surprise, their experiments revealed that this 1000 year old concoction did in fact have the desired antimicrobial activity they had hoped for. Not only was the recipe successful at eliminating bacterial infections in cell culture, the same result was similarly achieved in an infected mouse model. The results found the "ancientbiotic" to be particularly potent against single cell and bio-film forming MRSA infections. Surprisingly, Harrison's team also noted that it took longer for the bacteria to develop resistance against the antimicrobial soup. For the group and microbiologists globally, this is an exciting phenomenon, which could provide a promising new direction for antimicrobial agents.

Dr Freya Harrison (now University of Warwick) and Dr Steve Diggle (University of Nottingham), with the 'ancient soup' [credit: PA Wire].

Harrison's team investigated the significance of each component within the ancient recipe; despite their scepticism, they discovered that even the wine was an essential ingredient! They further showed that the presence of all the original ingredients were vital in successfully killing the bacteria. But what's so special about some leek, garlic,

ox gall and wine all tossed together and mixed in copper lined bowl? It sounds more like your Grandma's special stew recipe! What is it about these ingredients that bacteria don't like? Researchers hope that by identifying the potent chemicals which are effective against killing the bacteria that they can isolate the active components and incorporate them into new antimicrobial agents. These are desperate times. The golden age of antibiotics is over. The post-antibiotic era has dawned upon us and now is the time to diverge away from traditional methods and venture in a new direction.

We are embarking on a new quest to find antimicrobial agents to prevent bacterial infections launching a new defensive against mankind. To battle against resistant bacteria we must prepare to embrace novel strategies. No search can be too far or too wide: we are desperate for a solution to prevent the thick smog of infection re-surfacing from the past. However, no scientist could have imagined that the answers they were seeking could be concealed in ancient manuscripts. Perhaps now is the time to turn to the past for answers to the future...

References

1. World Health Organisation (2016). Antibiotic Resistance. http://www.euro.who.int/en/health-topics/disease-prevention/antimicrobial-resistance/antibiotic-resistance [last accessed 12/12/2016].
2. McAdam, P. R. *et al*. (2012). Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant *Staphylococcus aureus*. Proceedings of the National Academy of Science U S A. 109: 9107-9112.
3. World Health Organisation (2016). Antibiotic Resistance Fact Sheet. http://www.who.int/mediacentre/factsheets/fs194/en/ [last accessed 12/12/2016].
4. Harrison, F. *et al.* (2015). A 1,000-year-old antimicrobial remedy with antistaphylococcal activity. MBio. 6(4):e01129.

Malaria Bugs Outsmart Us Again; Will it be the Last Time?

Dafni Bechtsi

"I've heard mothers here say you have to have six children, so that you get one who will be educated and take care of you financially and one who will stay home and take care for you when you are old," said Mary Hamel, chief of the malaria branch at the CDC/Kenya Medical Research Institute. At this point you might well be asking yourself what happened to the other 4 children, and the sad truth is that they all, in fact, died from malaria. Indeed, by the time you finish reading this article, five more children in Africa will have died from malaria.

Malaria is a disease caused by the microscopic parasite, *Plasmodium*, transmitted from one person to another via the bite of a female mosquito. Last year alone, malaria killed 483 000 people and caused 214 million new cases. Of these, 91% were children under 5 years old and pregnant women in sub-Saharan Africa. Fever, chills, blurred vision, fatigue and body aches, all of which are common in "mild" malaria cases, keep children out of school and parents off work. In fact, malaria costs Africa $12 billion every year and even more in lost productivity[1]. Worryingly, the disease has recently returned to Europe, and experts warn that mosquitoes could potentially bring it to the UK as climate change kicks in[2].

A malaria-transmitting *Anopheles* mosquito taking a bloodmeal.

International efforts to defeat malaria have achieved a 60% decline in deaths over the past 15 years. Insecticide treated bednets, quick diagnosis and effective drugs have been the arsenal in this fight against the spread of this deadly parasite[1]. Now, you are probably thinking - it looks like we already have a number of effective drugs that do the job, so why did Bill Gates and George Osborne recently decide to invest £3 billion to support research towards developing new drugs and combatting malaria? The answer is simple; the *Plasmodium* parasite, just like any poor soul

Distribution of antimalarials in Italy in the 1930s.

being challenged in this dog-eat-dog world, has defended its species from our attack by resisting killing from existing anti-malarial drugs.

In the course of history, the malaria parasite has defanged, one by one, every single drug we have developed to kill it. In the 1950s, Chloroquine (CQ) was hailed the wonder drug that would eradicate malaria from the world, but within ten years of use, the parasite became resistant to it. This means that CQ no longer cleared out resistant parasites from the human body efficiently. CQ-resistant parasites were first spotted in Columbia and Thailand and quickly spread through Africa and most malaria-affected areas. The only alternative treatment at the time was a combination of Sulfadoxine and Pyrimethamine (SP), but that also encountered drug-resistant parasites only a year after its implementation. Several other antimalarial drugs have since been used to combat the *Plasmodium* superbugs resistant to CQ and SP, which (surprise-surprise!) have also resulted in the selection of resistant parasites in most malaria-affected countries[3].

Quinine hydrochloride tablets made by Burroughs Wellcome and Company, London [Science Museum A627532, photo no. L0058217].

The rise of drug-resistant parasites resulted in the highest malaria death rates on record, reaching over 1.8 million in 2004. The fresh line of defence against the *Plasmodium* superbugs became Artemisinin, a natural product isolated from sweet wormwood, a plant that has been used in China as a herbal remedy for centuries.

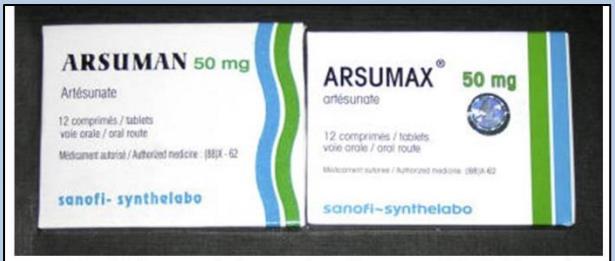

Sweet wormwood (*Artemisia annua*).

The drug acts as a quick but powerful punch that kills parasites in the patient's bloodstream. Its combination with another long-acting compound ensures there are no lingering survivors. Artemisinin-based combination therapies (ACTs) are currently the frontline treatment - and often the only one - against malaria infection. Since 2008, doctors in the greater Mekong sub-region in Southeast Asia have been reporting that ACTs take much longer to treat patients than usual. Alarmingly, in some areas, ACTs are now failing completely[4]. Patients are showing symptoms after treatment with what is considered our best weapon against *Plasmodium*. The parasite is fighting back once again.

It is sad, but true, that resistance to antimalarial drugs was inevitable. Drug resistance is not just a malaria-related phenomenon, and scientists have been well aware of its threat for decades. The phenomenon had been reported soon after the introduction of the first antimicrobials in 1937 and the same principle and mechanisms operate today. So how does antimicrobial resistance emerge? Every parasite, bacterium or virus in a population undergoes random mutations (small changes in their genetic code), many of which will be harmful or useless. But every now and then a single mutation comes along that makes a "lucky" microbe more successful. If that mutation makes it resistant to an antimicrobial, by preventing the drug target from being attacked or pumping the drug out of its system for example,

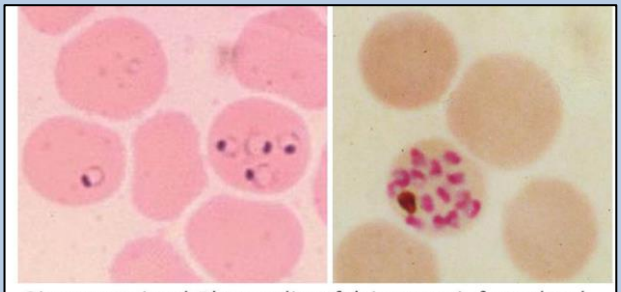
that gives it quite the edge! As its non-resistant mates are killed off by the drug, there is more room and resources for the resistant one to thrive, passing on to its heirs the mutations that helped it to do so.

If resistance was expected, why did we fail repeatedly to prevent its onset and why does it remain a threat? There is no doubt that humanity messed up here. Believe it or not, patients were given fake drugs or drugs that were out-of-date or did not contain enough of the ingredient that kills the parasites. Scientists at the National Institutes of Health estimated that about 35% of the antimalarial medicines in Southeast Asia were of poor quality and 36% were fake. Products labelled "artesunate" were found to contain no artemisinin-related compound at all. As if this was not bad enough, patients often received only one drug instead of combinations as advised or did not complete their course of treatment[5]. All these situations create a land-of-opportunity for the parasites. They are exposed to just about the amount of drug that will allow for the "lucky" resistant mutants (that can deal better with the drug) to take over and for antimicrobial resistance to emerge. You can imagine that poor diagnosis and delayed access to treatment in developing nations permits the unobstructed multiplication of rebelling parasites.



Genuine (right; see hologram) and counterfeit (left) packets of antimalarial Arsumax (Artesunate) from Cameroon [Photograph by Manuela Sunjio. Newton et al., (2006) PLoS Medicine 3:e197].

The tricky *Plasmodium* parasites do not make the situation any easier for us either; they are particularly successful in quickly spreading antimicrobial resistance, once it has emerged. Up to a trillion parasites can be found in a severely ill patient, each one of which replicates every 48 hours giving rise to 8-16 daughter parasites which inherit its genetic makeup. You can appreciate how quickly the "lucky" parasite can create a whole generation of "lucky" ones. Here's another titbit you should know: *Plasmodium* has a very high libido; it won't make the next move unless it has had sex. It is the only microorganism known for which every single transmission event is a result of a preceding "act of love", or more accurately, sexual recombination. In other words, they mate every time they pass through mosquitos, leading to an exchange of pieces of their DNA. These factors ultimately result in the rapid spread of mutations that help the parasite evade drugs[6]. Oh and… bloodsucking mosquitos pass these parasites from person to person and they certainly don't care where we draw our national borderlines.


Giemsa-stained *Plasmodium* falciparum-infected red blood cells. Left: ring stage; right: later schizont stage.

Now, taking into consideration your newly obtained knowledge on how antimalarial

resistance spreads and the fact that artemisinin resistance has so far spread in Cambodia, Laos, Thailand, Vietnam and Myanmar, you can decide for yourself whether you feel threatened by these little fellas or not. But remember, the main lesson we can learn from history, is that it repeats itself. Artemisinin resistance is likely to follow the same course seen in the past with previous antimalarial therapies, threatening to destroy all the efforts made so far to control the disease. This time however, we do have a new tool: genome sequencing. Using the latest technologies, we can read the sequence of every gene in the DNA of thousands of parasites, directly from patient blood samples. Comparing parasite DNA across the globe, we can spot specific mutations that cause artemisinin resistance and screen parasite populations for these genetic features. This way, we can track drug resistant parasites and keep ahead in the battle to control them. Following this tactic, researchers have now also detected artemisinin resistant parasites only 25 km off the Indian border[7] and, to put it in context, the last time antimalarial resistance spread in India, millions of people died.

Plasmodium parasites grew stronger as we grew careless. For almost a century, we played the game of leapfrog; our drug, their resistance, a new drug and then resistance again. The game now though seems to be coming to an end and we do not seem to be winning. This time there are no drugs that can satisfactorily replace artemisinin. There is a lot of fear of what will happen when artemisinin-resistance parasites make it over to sub Saharan Africa, where most malaria cases occur. How much time do we have before these superbugs take over? Will you feel threatened when we are facing an outbreak of untreatable malaria?

References

1. World Health Organization (2015). Factsheet: World Malaria Report 2015. Available from: http://www.who.int/malaria/media/world-malaria-report-2015/en/ [last accessed 12/12/2016].
2. Medlock, J.M., and Leach, S.A. (2015). Effect of climate change on vector-borne disease risk in the UK. *The Lancet Infectious Diseases*, 15: 721–730.
3. Petersen, I., Eastman, R., and Lanzer, M. (2011). Drug-resistant malaria: molecular mechanisms and implications for public health. *FEBS Letters*, 585: 1551–1562.
4. Plowe, C. V. (2014). Malaria: resistance nailed. *Nature*, 505: 30–31.
5. Nayyar, G. M. L. *et al.* (2012). Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa. *The Lancet Infectious Diseases*, 15: 488–496.
6. Davies, M. *et al.* (2014). Malaria eradication: can we do it? Should we do it?. In: Biology Week 2014. Recording [online]. London: The Royal Society of Biology. Available from: https://www.youtube.com/watch?v=0RqqumqInM [last accessed 12/12/2016].
7. Tun, K.M. *et al.* (2015). Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker. *The Lancet Infectious Diseases*, 15: 415–421.

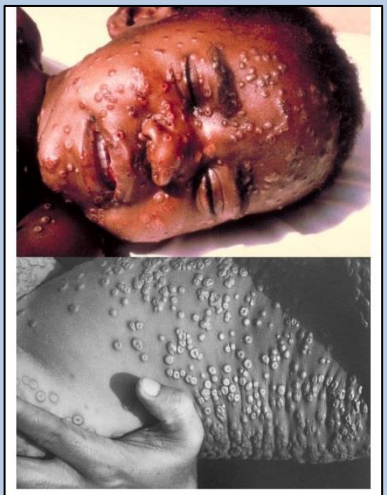
Eradication of an infectious disease

Total Wipeout

Skye Storrie

Nowadays, smallpox is a distant memory, slowly fading into the abyss. However, wind back through time and the pus filled lumps covering an individual's body would send a shiver down even the bravest person's spine. Smallpox was spread all over the world by traders from Africa, throughout Asia, Europe and the Americas. Millions of people succumbed to the disease and if it was not for the observations of a few farmers this may still be the case.

Smallpox is caused by the Variola virus. Bishop Marius of Avenches coined the name Variola from the Latin words for stained (*Varius*) or mark on the skin (*Varus*)[1]. There are 2 main types, Variola major and Variola minor. Variola major is the most common form, causing severe disease. Variola minor is less fatal, causing a milder form of the disease[2]. Pox is Latin for spotted and refers to the puss filled lumps that occur all over the face and body following infection. Transmission often occurs via close face-face contact. Contact with infected bodily fluids or heavily infected objects, like bedding, can also allow transmission. More rarely, the virus is transmitted through the air in confined spaces, such as in buildings or on public transport. The first symptoms of infection include fever, headaches, body aches, and sometimes vomiting. After a few days, small red spots appear on the tongue and in the mouth. Those little red spots transform into sores that erupt like a volcano, oozing virus into the mouth and throat. The person is now highly contagious. A rash starts to appear on the face, before spreading across the whole body. This rash becomes bumps filled with thick, opaque liquid. These bumps are often very distinctive with a small dimple in the middle that makes it clear that this poor soul is a victim of smallpox. These bumps transform, morphing into round and firm puss filled lumps. A crust forms on the lumps and these scabs start to fall off, leaving behind a nasty scar, a constant reminder of the suffering you endured. The person is infectious until every last scab falls off[2]. Not only were survivors left with disfiguring scars, some suffered from blindness as well[3].



Top: smallpox lesions on the face of a young boy (1969) [credit: CDC; PHIL ID#: 3]. Bottom: smallpox lesions on the torso of a 1973 patient in Bangladesh [credit: CDC/James Hicks; PHIL ID#: 284].

Smallpox, also known as the 'speckled monster', has shaped history. It is thought that this virus emerged as long ago as 10,000 BC. The earliest evidence of infection can be seen on Egyptian mummies bearing scars that resemble smallpox lesions (1550-1085 BC). These characteristic lesions can be seen on the face of the mummified

remains of the Egyptian pharaoh Ramses V. The Plague of Antonine that significantly weakened the Roman Empire is largely blamed on smallpox[4]. The introduction of smallpox to new areas during European colonisation led to more deaths than military conquest[3]. Natives had never been exposed to the virus and so had no immunity. This contributed to the fall of both the Aztec and Inca empires.

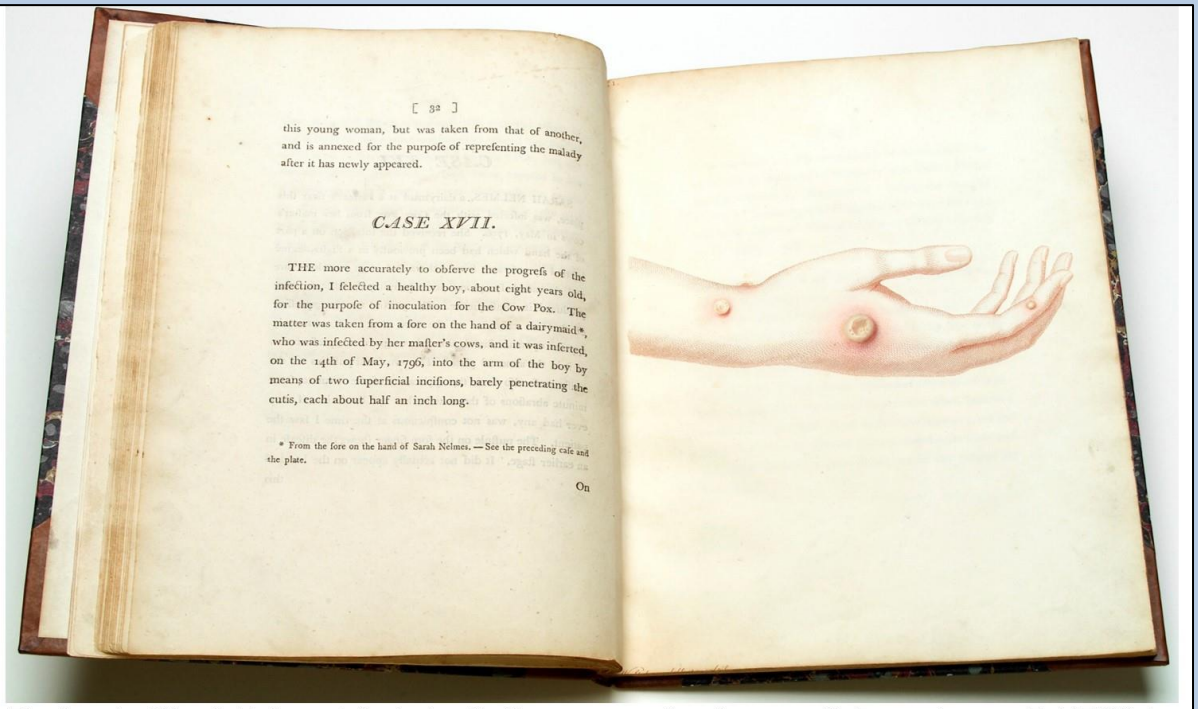
Smallpox was also utilised as a deadly weapon. Imagine strange foreign invaders entering your homeland. At first you are unsure; what do these men want? Then they come bearing gifts of clothing and blankets. You become slightly more trusting. However, little do you know these 'gifts' are laden with smallpox. Your friends and family become severely ill and many are killed. A brutal tactic, used by both the French and the British against the Native Americans. Smallpox was responsible for an astonishing 300 million deaths in the 20th century globally[3].

Many famous historical figures were wiped out by smallpox infection, including Queen Mary II of England, Tsar Peter II of Russia and King Louis XV of France, to name but a few[4]. Smallpox may even have shaped fashion trends. It has been suggested that Queen Elizabeth I of England resorted to a lead-based white cosmetic to cover her smallpox scars. Although this gave her a nice white complexion, this may in fact have lead to her death, excuse the pun.

Variolation against smallpox proved a successful means of protection against the disease. This entailed taking the contents of a ripe pus filled lump from an infected individual. The skin of the recipient would then be scraped and the contents of the pustule put under the skin[5]. The introduction of this practice to Britain is attributed to Lady Mary Wortley Montagu, the wife of the British Ambassador to the Sublime Porte in Constantinople[5]. She herself had survived a smallpox infection in 1713. Her brother had not been so lucky and had succumbed to the disease. Naturally, she did not want her children to suffer as she had or risk them facing the same fate as her brother. Whilst visiting Constantinople, Dr Charles Maitland variolated her son and on return to England her daughter so they became immune to smallpox infection[1]. Variolation would normally cause a mild case of smallpox and in many cases this rendered the individual protected from smallpox infection[5]. News of the success of this strange practice later reached the Royal family. In 1721, Charles Maitland was allowed to perform experiments on prisoners. This proved a success and the prisoners were shown to be immune to smallpox following exposure to infection. He then repeated these experiments on orphans just to be sure, which again proved a great success. Now convinced that the practice was relatively safe, Charles Maitland went on to variolate the daughters of the Princess of Wales. Although variolation proved a great success, it was undoubtedly a risky strategy and occasionally triggered outbreaks.

Lady Mary Wortley Montagu (after 1716) by Charles Jervas.

As time progressed, it was widely known that milkmaids who had contracted cowpox did not get smallpox. In 1774, Benjamin Jesty, a farmer from Dorset, infected his wife and two sons with cowpox to protect them against smallpox[5]. Although cowpox is caused by Vaccinia virus, a virus of cows, it is very similar to smallpox.


[32]

this young woman, but was taken from that of another, and is annexed for the purpose of representing the malady after it has newly appeared.

CASE XVII.

THE more accurately to observe the progress of the infection, I selected a healthy boy, about eight years old, for the purpose of inoculation for the Cow Pox. The matter was taken from a sore on the hand of a dairymaid *, who was infected by her master's cows, and it was inserted, on the 14th of May, 1796, into the arm of the boy by means of two superficial incisions, barely penetrating the cutis, each about half an inch long.

* From the sore on the hand of Sarah Nelmes. — See the preceding case and the plate.

On

The hand of Sarah Nelmes infected with the cowpox [book opened]. From: Jenner, E. (1798) An inquiry into the causes and effects of the Variolae Vaccinae [credit: L0043483 Wellcome Images].

Undoubtedly the most famous smallpox experiments were conducted by the physician Edward Jenner. A dairymaid called Sarah Nelmes came to Jenner with lesions on her hands. Jenner quickly diagnosed this as cowpox and seized the opportunity to test the protective properties of a cowpox infection against a subsequent smallpox infection. Of course you don't want to be conducting such risky experiments on yourself. Therefore, the most logical thing to do is to take the 8 year old son of your gardener (James Phipps) and infect him with cowpox, then subject him to a smallpox infection. Risky strategy right? However, to both Jenner and I'm sure Phipps relief, these experiments yielded fruitful results. Phipps developed a mild illness following smallpox infection but was now immune. Despite the fact that Edward Jenner was not the first to suggest or attempt to utilise the protective properties of cowpox

Edward Jenner performing his first vaccination on the 8 year old in 1796 [credit: Ernest Board (1877-1934)].

against smallpox, he is largely credited with this achievement. Unlike Jesty, he published his findings in a book, and thus, vaccination was born[6]. This knowledge was the driving force behind the eradication of smallpox.

It was not until many years later, in 1967, that the World Health Organisation launched the intensified Smallpox Eradication Programme. Smallpox was still having a significant impact worldwide, with 10-15 million cases a year. 465 million doses of vaccine were donated by 27 countries to help the effort. The last natural case of smallpox was recorded in 1977, in Somalia. However, in 1978, a laboratory accident led to a small outbreak of smallpox in Birmingham, causing the death of one person. This was the last ever case. The World Health Assembly triumphantly declared that smallpox had been successfully wiped out on the 8th of May 1980[4]. This marked a huge milestone in the history of disease control. This was the first time that any disease had been eradicated, giving hope to all in the global fight against infectious diseases.

References

1. Riedel, S. (2005). Edward Jenner and the history of smallpox and vaccination. In: *Bayer University Medical Center Proceedings*, 18: 21–2.
2. Centers for Disease Control and Prevention (2016). Smallpox Fact Sheet: Smallpox disease overview. Available from: https://emergency.cdc.gov/agent/smallpox/overview/disease-facts.asp [last accessed 12/12/2016].
3. World Health Organisation (2011). Smallpox. Eradicating an ancient scourge. In: Bugs, drugs and smoke: stories from public health. pp. 3-21. World Health Organisation, Geneva, Switzerland.
4. Fenner, F. *et al.* (1988). Smallpox and its eradication. *History of International Public Health* 6, World Health Organisation, Geneva, Switzerland.
5. Taylor, M.W. (2014). Smallpox. In: Viruses and man: A History of Interactions. pp. 143-162. Springer International Publishing Switzerland.
6. Jenner, E. (1798). An Inquiry into the causes and effects of the Variolae Vaccinae; a disease discovered in some of the Western counties of England, particularly Gloucestershire, and known by the name of the Cow Pox. Sampson Low, London, England.

In the rinderpast

Sara Elg

Have you ever heard of rinderpest? How about cattle plague? If you have, it probably was a while ago. It has been five years since this disease was officially declared eradicated, doubling the number of diseases successfully eradicated by humans. Prior to May 25th, 2011 only smallpox, a horrible disease of humans, had been eradicated[1,2]. Another eradication, great news! Except it is not really news. This happened five years ago, why should we care now? In fact, if it only affected cattle, should we care at all?

In the world today, there is an on-going debate surrounding the use of vaccinations. Some groups claim that vaccines can be dangerous and do more harm than good. In some areas, this has led to a drop in vaccinations, and in turn to outbreaks of diseases such as measles[3]. In contrast to rinderpest, measles is probably more familiar to you. Everyone has heard of it, but thanks to vaccinations, most of us have not had it. Rinderpest and measles actually have a lot in common, and perhaps the story of how we eradicated rinderpest can remind us just how important and good vaccines can be.

So, what was rinderpest? Well, the disease was an infection of cattle and buffalo, as well as wild animals like antelope, deer, giraffes and wildebeests[2,4]. Infected animals suffered from fevers, a loss of appetite and diarrhoea, as well as terrible blisters around the mouth and nose[4]. Although some animals eventually recovered, they were a minority. The disease had a very high mortality rate, even up to 100% in some outbreaks[5]. All this was caused by a virus very similar to the measles virus[2]. The most well-known and devastating outbreak occurred in southern Africa in the 1890s, as almost 90% of all cattle was killed[2]. Cattle are used to plough and fertilise fields, and the huge loss severely affected harvests. The disease also had major effects on wild-life, even leading to lions desperate for food attacking humans[2,5].

Rinderpest outbreak in South Africa, 1896.

Historically, the approach used against the disease was similar to what is nowadays used for outbreaks of foot and mouth disease: culling of infected and exposed animals[4]. As you can imagine, this approach was not too popular back then either! It was known that animals that recovered from infection could never get rinderpest again, so research began into what we now know as vaccination. This research led to many important scientific discoveries that inform the design of vaccine strategies

even today. For example, the role of maternal protection through mother's milk was discovered through experiments involving the application of material from rinderpest-recovered animals to calves, and in the late 1800's, it was discovered that serum from recovered animals could protect unexposed animals[2].

Injection of serum together with virus became the first vaccination; however, this seemed to spread other diseases. As a replacement, virus passed through goats before injection into cattle was later used to protect animals[2]. This technique made the virus less dangerous, and actually passaging virus to make it harmless before using it in vaccines is a common method in the vaccination manufacturing process even nowadays.

Community animal health worker vaccinating animals against rinderpest in Uganda.

The vaccine for rinderpest was perfected by Walter Plowright in the 1950's, and it was very similar to the measles vaccine[2]. Vaccinated animals were immune for life and the vaccine was safe, cheap and easy to make[6]. However, to distribute the vaccine in rural areas of Africa and Asia, it had to be made tolerant to heat. Fortunately, the technique of freeze-drying had recently been discovered, and could be used not only for space food, but to increase the shelf-life of the rinderpest vaccine by several years[6].

The vaccine was extremely successfully used across the globe to reduce numbers of rinderpest cases. In 1976, only three countries reported cases of the disease. However, the world's cattle population was now more susceptible than ever, as vaccinations declined and funds for disease control was diverted. This was proved in the 1980's, as an outbreak spread across Africa from Sudan, killing millions of animals, both cattle and wildlife[6,7].

In response to the outbreak, the Pan-African Rinderpest Campaign (PARC) began in 1987, aiming to control rinderpest through vaccinations, and by 1990 cases of rinderpest were only reported from four African countries[7]. In 1992, the United Nations Food and Agriculture Organisation (FAO) stated that global eradication would be possible, and economically beneficial[7]. A collaboration with the World Organisation for Animal Health (OIE) was set up and in 1994 the Global Rinderpest Eradication

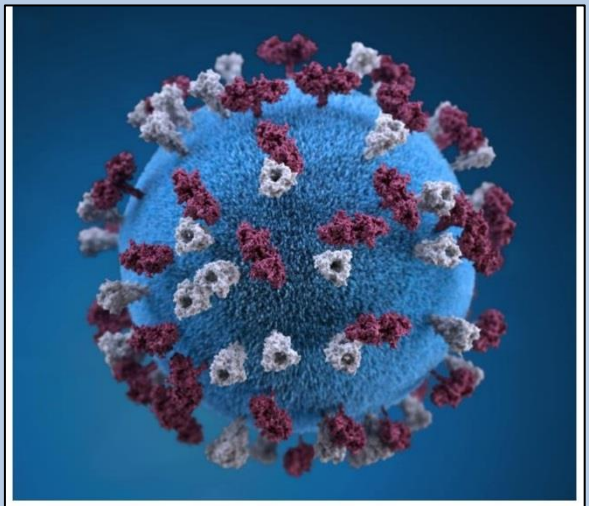
Poster produced by PARC/Ethiopia (in local language Luganda) to advise livestock owners on detecting and protecting against rinderpest.

Programme (GREP) was initiated. This programme linked regional campaigns and had as a goal to achieve global eradication by 2010, using mass vaccinations and closely controlled monitoring of cases[7].

In 2001, the last confirmed rinderpest case globally was reported from Kenya, and in 2009 Kenya was declared free from rinderpest completely[1,4]. The last vaccinations globally were given in 2006, and in 2009 surveillance operations ceased as no evidence of disease could be seen[8]. By June 2011, when the official eradication declaration was held by the United Nations, 198 countries had been freed from the burdens of rinderpest.

A lot was learned over the course of this eradication campaign, lessons that have informed our current research into vaccines and disease control and eradication efforts. For example, the importance of restricting animal movements and isolating infected animals and areas has helped form responses to foot and mouth disease outbreaks[4]. The history of this eradication also clearly shows us the devastating effects of complacency when it comes to vaccinations. The resurgence outbreak in Africa in the 1980's killed millions of cattle as a consequence of relaxed control efforts and a decline in vaccinations. Recently we have seen an increase in measles cases[3] as vaccination rates have dropped, perhaps as an effect of anti-vaccination advocacy or complacency. Hopefully this will trend will change and not result in any larger outbreaks.

Perhaps most importantly, the successful campaign for the eradication of rinderpest has shown that eradication is possible, through dedicated international cooperation toward a common goal. Several other eradication campaigns are underway globally at the moment, for diseases like polio and dracunculiasis (or guinea worm disease), and also for malaria. With rinderpest as an example in particular, perhaps it would also be possible to start thinking about the eradication of measles? Measles and rinderpest are very similar viruses, with clear symptoms and vaccines that can protect against disease for a long period of time. Unlike rinderpest, measles also only has one host: it likes us humans! All of these facts mean one thing: if we just put our mind to it, we could eradicate measles. All this from learning about our rinderpast...



3D graphical representation of a measles virus particle. The maroon studs (H-protein) bind the virus to host cells while the grey studs (F-protein) enable the virus to enter host cells [Credit: CDC/Allison M. Maiuri, MPH, CHES; Illustrator: Alissa Eckert; PHIL ID#:21074].

References

1. Fournié, G. *et al.* (2013). Rinderpest virus sequestration and use in posteradication era. *Emerging Infectious Diseases*, 19: 151-153.

2. Morens, D. M. *et al.* (2011). Global rinderpest eradication: lessons learned and why humans should celebrate too. *The Journal of Infectious Diseases,* 204: 502-505.

3. Centers for Disease Control and Prevention (2016). Measles Cases and Outbreaks. Available at: http://www.cdc.gov/measles/cases-outbreaks.html [Last accessed 12/12/2016]

4. OIE (World Organisation for Animal Health) (2016). General Disease Information Sheet - Rinderpest. Available from: http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/RINDERPEST-EN.pdf [last accessed 12/12/2016].

5. Hamilton, K. *et al.* (2015). Identifying and reducing remaining stocks of rinderpest virus. *Emerging Infectious Diseases*, 21: 2117-2121.

6. Taylor, W. P. *et al.* (1995). The principles and practice of Rinderpest eradication. *Veterinary Microbiology*, 44: 359-367.

7. Rweyemamu, M. M and Cheneau, Y. (1995). Strategy for the global rinderpest eradication programme. *Veterinary Microbiology*. 44: 369-376.

8. Food and Agriculture Organisation. The Global Rinderpest Eradication Programme. Progress report on rinderpest eradication: Success stories and actions leading to the June 2011 Global Declaration. Available from: http://www.fao.org/ag/againfo/resources/documents/AH/GREP_flyer.pdf

Exploiting endosymbionts to control infectious disease

Microbial Friends

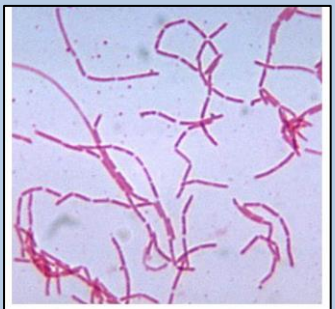
Imtithal Al Kindi

Imagine zooming-in inside your body. There you see a world of bacteria, a high density population of small organisms from all different colours and shapes. All working together, hand-in-hand, to build their empire. It is indeed a busy world. They are everywhere; your skin, mouth, stomach and even in your urinary tract! You, together, promise to be true to each other, in good times and in bad, in sickness and in health until death do you apart.

These bacterial populations are known as the microbiota. It is not so long ago that scientists discovered that humans harbour a dense population of friendly bacteria, about 100 trillion bacterial cells! Contrary to the bad press that bacteria get in soap adverts, this microbiota relationship with humans is a bargain in which each part benefits from the other. The microbiota provide essential amino acids and vitamins that you can't make yourself. They are indeed good friends because they also aid you with food digestion. Most importantly, they serve as managers of cells of the host immune system – Humans' security system. Additionally, they form a defence barrier from evil bacteria of the outer world. Think of them as guardians of humans. And in return, humans provide a loving home and food for the microbiota community.

You may be wondering where all these bacteria come from. In fact, humans acquire these during birth and the first months of life. You make friends with good bacteria every time you're exposed to one. It could be from the hospital or from your mother's loving kisses! As you grow older, your collection of microbial friends becomes complete.

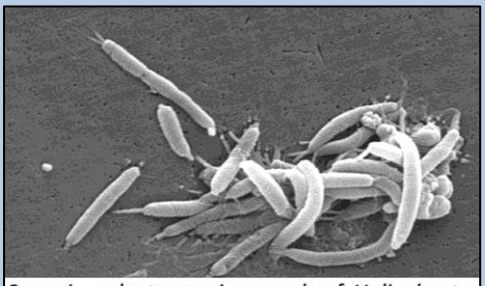
Who would have thought that exposure to microbes could be beneficial? Recent studies suggest that human interaction with microorganisms, especially in early life, could train your immune system to combat and fight pathogenic bacteria or diseases in the future. Our friendly bacteria monitor our body sites and protect us from being colonized by bad bacteria. They can do that by three main smart strategies: either by occupying all the available regions so there is no space left for the bad guys, or by using weapons like antagonistic substances, or by taking over all the food and nutrients available, leaving the bad bacteria starving to death.



Light micrograph of *Bacteroides* bacteria [credit: CDC/Dr V. R. Dowell, Jr.; PHIL ID#:2953].

Residing within the intestine is a large community of friendly organisms. Gut microbiota secrete defence molecules that protect humans from colonizers like *Enterohaemorrhagic E.coli*. Moreover, Bacteroidetes members are important for restoring proper immune balance and function to

modulate infection responses in the gut. For example, *Bacteroides fragilis* can fight *H. hepaticus* and prevent inflammatory bowel disease[1].



Scanning electron micrograph of *Helicobacter* cells. [Credit: CDC/Dr Patricia Fields & Dr Collette Fitzgerald; photo credit: Janice Haney Carr]

Another champion is *Helicobacter pylori;* this infantry lives in the stomach and if you are lucky enough, it can protect you from Tuberculosis, an infection that leaves you breathless with endless coughing and chest pain. Although the secret weapon of *H. pylori* is not yet identified, it seems to constantly send warning signals to the immune system[2].

However, it is not always rainbows and butterflies, rules and conditions apply. The world of the microbiota is peaceful, however when imbalance strikes, it can be a mess!

Studies have shown that excessive use of antibiotics or high-fat diets can shift the balance of the microbial communities favouring one community over others. Prolonged antibiotics exposure and poor diet can sadly kill some of our microbial friends. This unbalance can create a disturbance in the gut. Think of this as a competition. Bacterial communities that are larger in numbers will outcompete the smaller communities by consuming more nutrients and occupying more niches. Bacteria are selfish, once their environment is favourable, they become greedy and dangerous. That's when they win the competition and establish an infection.

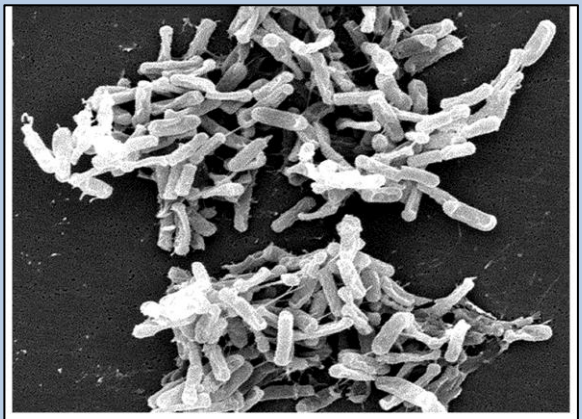
Gastrointestinal infections such as irritable bowel syndrome, Crohn's disease and *Clostridium* infection are the outcome of an imbalance between the number of good and bad bacteria. So, how can we exploit our relationship with the microbiota for the purpose of protection from infections?

Since our faeces originate from food being digested in the gut and processed in the intestine then it would make perfect sense that our poo carries a troop of our friendly bacteria. Scientist explored faecal bacteria composition and thought of turning feaces into something nice and useful instead of being a stinky useless bulk.

You may have heard about organ transplants like heart, kidney and skin, but what about faecal transplants? Sounds disgusting right? But you'll be crying for this when you have a gastrointestinal infection and no antibiotic in the world can cure you. In fact, the idea of planting faeces in humans and animals for the purpose of re-establishing the balance of the microbiota is quite old deriving from the late 1950s. Most importantly, donors for faecal transplantation can be anyone, even a total stranger as long as the donor is healthy. Even better, someone else's poo can save you in 93-98% cases! The procedure involves collecting faecal matter from a healthy donor, purification, mixing with saline solution and delivering it to the patient, usually by colonoscopy[3,4].

Faecal transplants are currently being proposed for patients who suffer from

recurrent *Clostridium* infection. The infection is characterized by diarrhoea and severe abdominal pain. It occurs mainly in hospitalized people due to prolonged exposure to antibiotics. *Clostridium difficile* infections are killing thousands of people worldwide annually. The Centers of Disease Control revealed 500,000 cases of the infection in 2012 in the United States alone with 14,000 deaths reported.



Scanning electron micrograph of *Clostridium difficile* bacteria [credit: CDC/Lois S. Wiggs; PHIL ID#: 6260].

"Come on, you're taking the mickey" said Len Barnes, a 75-year-old patient with *Clostridium* infection after his doctor suggested a faecal transplant. Len Barnes suffered from a long-lasting *C. difficile* infection accompanied by loss of appetite and weight. After the failure of many attempts to clear the infection, a faecal transplant was done[5].

"My doctor explained that mixing healthy poo with my poo - and transferring it back into my bowel mixed with warm water - would give someone's healthy bacteria the chance to fight with my bad bacteria". The following day Mr. Barnes recovered and was discharged from the hospital[5].

And if you don't fancy a poo implant, here is some other good news: You can introduce new microbial friends to the gut by consuming probiotics rich foods (food rich with live good bacteria) e.g. yoghurt or by taking probiotic supplements. This super-food can restore the balance of your microbial community in the gut, but it may take longer than a faecal transplant. Nevertheless, an average serving of probiotics can only give you 1-30 members from the bacteria family but in poo, there are around thousands of different members from all over the world (of bacteria)[6]. Hurrah for poo!

Remember that rules and conditions apply in this battle between the good guys and the bad guys. This is best understood by thinking of it as a bargain. You give and you receive, but if you don't give by being the good host, then don't expect the guardians to stick to the agreement. Your state of nutrition, antibiotic intake, being ill or stressed could all break the agreement between you and your microbiota and it will lead to a battle of disease.

But it is good to know that with new research done every day on the benefits of good bacteria in preventing and curing various infections, our relationship with our little microbial friends can only prosper. Studies on faecal transplants revealed a promising future for not only *Clostridium* infections, but also *Candida* fungal infections, inflammatory bowel syndrome, colitis and even autoimmune diseases. Thank you our microbial friends!

References

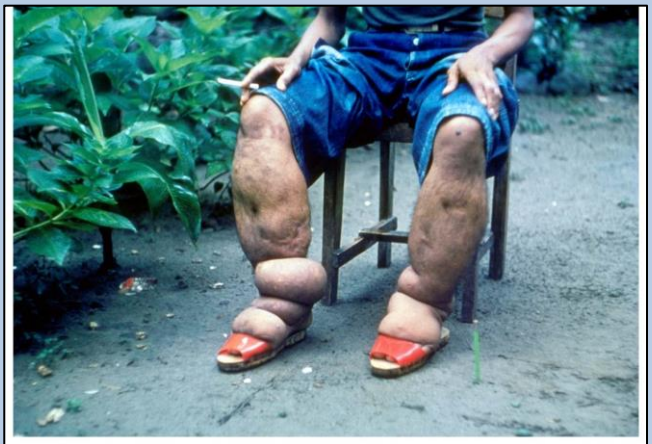
1. Round, J. L., & Mazmanian, S. K. (2009). The gut microbiome shapes intestinal immune responses during health and disease. *Nature Reviews Immunology*, 9: 313–323.
2. Clemente, J. C. et al. (2012). The impact of the gut microbiota on human health: an integrative view. *Cell*, 148: 1258-1270.
3. Brandt, L. J. (2012). Fecal transplantation for the treatment of *Clostridium difficile* infection. *Gastroenterology & Hepatology*, 8: 191–194.
4. Weingarden, A. et al. (2015). Dynamic changes in short- and long-term bacterial composition following fecal microbiota transplantation for recurrent *Clostridium difficile* infection. *Microbiome*, 3: 1-8. doi:10.1186/s40168-015-0070-0
5. Parry, L. (2015). Daughter's poo cures father of life-threatening infection: Pensioner struck by superbug undergoes faecal transplant to eradicate the illness. *Daily Mail* [online]. Available from: http://www.dailymail.co.uk/health/article-3033514/Daughter-s-POO-cures-father-life-threatening-infection-Pensioner-struck-superbug-undergoes-faecal-transplant-eradicate-illness.html [last accessed 12/12/2016].
6. Dr. Axe Food is Medicine (2012). The fecal transplant: can it help colitis, Candida, IBS and more? Available from: http://draxe.com/fecal-transplant/ [last accessed 12/12/2016].

The Hidden Weapon

Natalia Cameron Ruiz

When Goldilocks breaks into the three bears' house, she eats the bears' porridge and sleeps in their beds. She takes and takes but gives nothing in return. This is how certain bacterial infections work. The bacteria enter your body, causing an infection, and they take your nutrients until they are caught and expelled. But what if Goldilocks brought with her necessary materials for the bears to survive and even flourish while, in exchange, the bears gave her shelter and sustenance. The *Wolbachia* bacteria do just that. They infect insects, spiders and worms and hide inside their cells, but rather than harming them, they are essential for their survival. Among their hosts are a group of tiny parasitic worms which can infect humans and can cause up to 17 different diseases, making them a great burden to society. The *Wolbachia* bacteria are our hidden weapon against them[1].

These nearly microscopic parasites worms are transmitted by insects and infect 150 million people in more than 80 tropical countries every year. More than 1 billion people are at risk of infection and it has been estimated that up to one in six people are affected by them in some areas. One of the most important diseases caused by one of these parasites is river blindness, also called onchocerciasis, which affects 37 million people[2]. The parasitic worm travels to the eye and causes people to become blind. If the infection is chronic the blindness can become permanent. Another disease is lymphatic filariasis, which causes long term disability in many people across Africa, with symptoms of elephantiasis. This is a huge swelling of arms or legs and a thickening of the skin, which mean they resemble an elephant's leg.

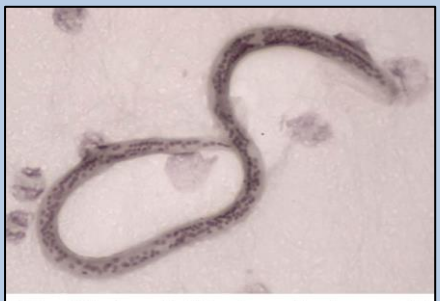


Elephantiasis of leg due to filariasis. Luzon, Philippines, 1962 [credit: CDC/PHIL ID#: 373].

There is no vaccine available at the moment for these worms but some of our drugs are still effective against them. You might have heard of bacteria becoming resistant to many of our antibiotics – this has become a great health concern worldwide. Imagine a herd of wildebeests in Africa crossing a river. There are hundreds of crocodiles waiting for them there. As they cross they are picked off one by one, but some always survive… The strongest, the fastest or just the luckiest! Antibiotics are the crocodiles that we use to kill bacteria. It's all more complicated than that, of course. Antibiotics are much more

effective than crocodiles and even if some bacteria survive they might be picked off by our immune system, the lions waiting on the other side of the river. But due to poor management of antibiotics and just good old evolution many bacteria can now cross the river with no fear of being eaten. Similarly, these parasitic worms are starting to be able to survive our treatments. Plus, drugs only kill the larvae, meaning that adult worms can live inside humans for up to 15 years, causing disease and having more offspring. So, as a result, treatments have to be administered regularly to try to keep up with new worms appearing, and the whole community has to be treated to prevent a massive epidemic.

Because of all this, we need new ways to defeat worm infections, and one of most interesting targets is the hidden *Wolbachia* bacteria living within them. These bacteria are extremely important for the worms; they are friendly Goldilocks. She is there when the worms are eggs, helps them grow correctly and stays with them through adulthood, assisting them by making essential products that allow the worms to survive. *Wolbachia* are also involved in inflammation, which is what causes most of the worm infection symptoms, from the swollen limbs to the blindness. The bacteria from the worms can get into the patient's body where their immune system tries to fight them. Their body fires everything it has against them, even allowing more blood to travel to the site where the worm is living. Blood contains many cells that are designed to fight infection. This leads to the external sign we call inflammation, which is a great weapon, but it can lead to some seriously harmful consequences. For example, if the worm is in the eye, as occurs with river blindness, the eye gets so inflamed it loses its function and the patient becomes blind. *Wolbachia,* friendly Goldilocks, can also help the worm fight the patient's inflammatory response. Our immune system is a complicated machine; it has different answers to different situations. If a virus is hiding inside our cells, it has to be fought differently from worms happily swimming in our blood. The *Wolbachia* take advantage of this by attracting the wrong type of immune cell, which is incapable of killing the worms, while keeping the effective ones away. So we get horrible swelling and yet the worms still happily roam about. If we can somehow kill these hidden bacteria, if we murder Goldilocks, the worms, which need them to survive, will die too. We have found our weapon.



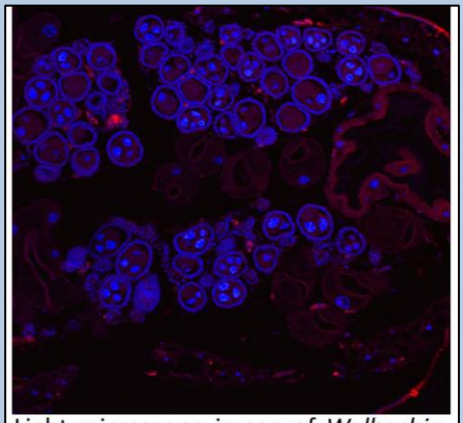
Microfilaria of *Wuchereria bancrofti,* one of the causative agents of elephantiasis (lymphatic filariasis), in a blood smear. [Credit: Marc Perkins]

Therapy to eliminate *Wolbachia* has focused on different antibiotic treatments[3]. The antibiotic kills the bacteria and leads to the death of the worms, even the adult ones that other drugs cannot kill! For example, in 2008 it was discovered that a 6-week treatment with an antibiotic, tetracycline, made female worms incapable of having offspring and even killed 60% of them. We can use these antibiotics to treat the whole community and kill the worms, effectively treating the disease. The absence of *Wolbachia* also leads to a more effective immune response against the worms

because the host can fight back properly as, if the bacteria are absent, the correct immune cells can show up and kill the worm. However, all the antibiotics that have been shown to work so far have side effects and cannot be used in young children or pregnant women. So, researchers are trying to find new ways to kill *Wolbachia* that are more effective and safer to use.

The discovery of new ways to target *Wolbachia* bacteria is picking up steam. Scientists are testing many different drugs and substances to find the best ones to kill bacteria. They are also trying to identify proteins, the building blocks of the cell, and genes, the instruction manual, in the bacteria that they need to survive. For example, if they find something that blocks them from constructing what they need to divide in two, they will eventually die out.

As *Wolbachia* bacteria are also found in insects, scientists are trying to use them as a strategy to stop the spread of diseases such as Malaria or Dengue fever[3]. They treat the mosquitos and then the parasite cannot travel within them and infect other people. There are a few strategies to do this. The first just reduces *Wolbachia* populations. For example, male insects with no bacteria, or the wrong kind of bacteria, are released into the environment, where they mate with females. The eggs will have problems developing, as Goldilocks is absent to guide them through. As the eggs cannot become insects their numbers will dwindle. The second method is called population replacement. In this strategy, females are the ones released with the wrong kind of bacteria. As *Wolbachia* pass from mother to offspring, the bacteria will be passed along to the next generation. These 'wrong' bacteria make the insect resistant to infection by other parasites. For instance, mosquitos infected with a modified *Wolbachia* strain are less likely to transmit the Dengue virus. If we take away their vehicle, the parasites cannot travel form person to person.



Light microscope image of *Wolbachia* bacteria (red) in mosquito tissue (blue: mosquito DNA) [credit: Jason Rasgon, Penn State].

We can now use the hidden *Wolbachia* bacteria, the helpful Goldilocks, as a target. We can kill them or change them, instead of having to attack the parasites harbouring them, giving us more potential treatments with fewer side effects.

References:

1. Slatko, B. *et al.* (2014). *Wolbachia* endosymbionts and human disease control. *Molecular and Biochemical Parasitology*, 195: 88-95.
2. Bouchery, T. *et al.* (2013). The symbiotic role of *Wolbachia* in Onchocercidae and its impact on filariasis. *Clinical Microbiology and Infection*, 19: 131-140.
3. Bourtzis, K. *et al.* (2014). Harnessing mosquito–*Wolbachia* symbiosis for vector and disease control. *Acta Tropica*, 132: S150-S163.

Contact

Dr Tansy C. Hammarton,
Institute of Infection, Immunity & Inflammation,
University of Glasgow,
120 University Place,
Glasgow G12 8TA.
Tel: +44 141 330 6766
Email: Tansy.Hammarton@glasgow.ac.uk
Web: http://www.gla.ac.uk/researchinstitutes/iii/staff/tansyhammarton/
Twitter: @Hammarton_lab

The School of Life Sciences,
University of Glasgow,
Bower Building,
University Avenue,
Glasgow G12 8QQ.
Tel: +44 141 330 3994
Email: lifesci-enquiries@glasgow.ac.uk
Web: http://www.gla.ac.uk/schools/lifesciences/
Twitter: @UofGlasgow