Sleeper cells

Our strongest antibiotics are being thwarted by bacteria that lie dormant, only to bounce back unharmed once the pressure is off. Ed Yong investigates
A KE a lawnmower to a field of grass and, with the blades on the right setting, you can raze it to the ground. It might look brutal, but all you are removing is the leaves. The roots remain. After the mower is gone, the plants start to grow again and within a few days, the field is back.

It now looks as if bacteria have a similar trick up their sleeve. A growing list of microbes have been found that are capable of entering a dormant state where all growth and reproduction ceases. Thanks to this trait, which is sometimes dubbed “persistence”, they can tolerate extremely high doses of antibiotics without becoming resistant in the normal sense. In classic antibiotic resistance, bacteria keep on growing even in the presence of a drug thanks to specific defence mechanisms, such as pumping the antibiotic out through their cell walls. Persistent cells simply play dead – they “keep their heads down” and stop multiplying, only to bounce back to life when the antibiotic has gone.

The distinction is important, because persistent cells need an entirely different design of antibiotic. And some medical problems that are seen as being due to antibiotic resistance may in fact be caused by persistence. In some cases, persistence raises the chance that bacteria evolve resistance, because you need to take antibiotics for longer to destroy all the persisters, as happens with throat infections, acne and tuberculosis (TB).

Yet ways of conquering bacterial persistence are in development and some may be about to reach the clinic. “There’s momentum building,” says Kim Lewis a microbiologist from Northeastern University in Boston, Massachusetts. “I think it’s ripe for exploding.”

A new strategy for tackling bacterial diseases can’t come soon enough, because the microbes have evolved ways of shrugging off virtually all antibiotics. To make matters worse, the production of new drugs has slowed to a crawl; although 15 classes of antibiotics were developed between 1940 and 1962, only three new classes have emerged since.

Persisters were first noted in 1944, just as penicillin was being mass-produced. Joseph Bigger, an Irish doctor, found that the drug could not completely kill a culture of Staphylococcus aureus, a bacterium commonly found on human skin that can cause a range of problem infections. Every time Bigger exposed the microbes to the drug, a small proportion survived to fight another day and yet, curiously, tests showed that they were not penicillin-resistant.

Bigger’s findings were largely ignored, but in the past decade a growing number of researchers have focused on this phenomenon. Every species of bacteria they have cared to test is capable of forming persister cells, which either do not grow at all, or else grow infinitesimally slowly.

What is going on? It seems that this dormant state evolved to allow bacteria to cope with harsh environments, such as low oxygen levels, lack of nutrients or adverse temperatures. Today, it allows them to shrug off antibiotics.

Lewis describes them as the ultimate adversary. “Their function is to be not killed,” he says. “They don’t do anything else. They don’t grow. They don’t propagate. They don’t do anything except survive.”

The untouchables

Depending on the context, the slumbering cells may be called latent, dormant, non-multiplying or tolerant. Whatever their name, they stay alive because antibiotics can’t touch them. All current drugs have been designed to kill bacteria that grow and multiply, interfering with the chemical reactions that make new proteins, or DNA.

“Multiplying cells are like machines with lots of moving wheels and cogs,” says Anthony Coates, a microbiologist at St George’s, University of London. “When you put in an antibiotic, it’s like a spanner going in the works. You get huge damage and the cell dies.” But if the bacteria go to sleep: “You have the same cogs but they’re stationary, so when you put in your spanner, nothing happens.”

Persisters are frustratingly difficult to study: everything about them defies the ways we normally study bacteria – by providing food and watching them grow. It is hard to isolate persisters because they are usually heavily outnumbered by normal bacteria. Looking for cells that grow in the presence of antibiotics won’t work because they do not grow.

What’s more, if you try to investigate persisters by injecting them into an animal or a new culture medium, they have an annoying tendency to wake up. “Almost anything you do by standard approaches, you fall into a trap,” says Lewis. “People
“About half of infectious diseases that are difficult to treat with antibiotics are not due to resistance but persisters”

have tried to study persisters for a year or two, got nowhere, and pulled out.”

Studying the genetics of persistence has been equally fraught. The obvious experiment would be to cause random mutations, look for cells that have lost their ability to turn into persisters, and so work out which genes are responsible. “Several labs including mine have put a lot of energy into this, but it has failed,” says Lewis.

It now seems that there are many paths to latency. Block any one of them, and bacteria simply opt for a different route. You can knock out almost every gene in the gut bacterium Escherichia coli, one by one, and all the resulting mutants will still form persisters. “There is a lot of redundancy in the system,” says Alex O’Neill, a microbiologist at the University of Leeds in the UK. “There are still large gaps in our understanding of what persisters are and what they’re doing.”

Fortunately, those gaps are starting to be filled, thanks to growing awareness of the big medical problems they cause. Take TB, one of humanity’s oldest and deadliest foes. While people with an active infection have fevers and cough up blood, a third of the world’s population have a more benign version known as latent TB. People with this form can go their whole lives without symptoms, only becoming sick if the infection reactivates, perhaps because their immune system weakens. It now seems likely that these latent infections involve dormant persister cells.

Although we have long known about latent TB, it is only in the past few years that we have discovered a persister mode in many other kinds of dangerous bacteria. Take Pseudomonas aeruginosa, a common bacterium in the environment that can cause pneumonia and wound infections. People with cystic fibrosis, a genetic disorder that prevents the lungs from clearing away mucus, usually die from pneumonia caused by this bacterium.

It has been a puzzle that antibiotics do little for these patients, even though the bacteria infecting them are susceptible to the drugs when tested in the lab. But in 2010, Lewis’s group found that the problem is really down to persistence (Journal of Bacteriology, vol 192, p 6191). “It has nothing to do with resistance,” he says. “What kills the patient is the ability to make dormant cells.”
Resistance or persistence – what’s the difference?

Almost all bacteria can thwart antibiotics by forming persister cells, a subtly different phenomenon from antibiotic resistance.

Antibiotic treatment

The vast majority of antibiotics can only kill cells that are actively growing and multiplying.

Bacteria may evolve antibiotic resistance through specific defence mechanisms, such as a molecule (blue) that pumps the antibiotic out of the cell, allowing the bacteria to replicate.

Persisters

If bacteria become persisters, they simply switch off, go to sleep, and cease multiplying.

When the antibiotic has gone, the bacteria wake up again and start multiplying.

It is hard to work out the scale of the problems caused by bacterial persistence, because no one has collated hard figures. But Lewis for one takes it seriously. “My estimate is that about half of infectious diseases that are difficult to treat with antibiotics in the developed world are not due to resistance but to persisters,” he says.

Another perspective is that more than 80 per cent of bacterial infections involve biofilms. If their tenacity is indeed usually down to persister cells, that is surely a reason to start paying them more attention.

So how do we take on the persisters when all the antibiotics we have were designed with multiplying bacteria in mind?

By chance, just a handful of our existing antibiotics work against persisters, although not well and their mechanism is often unclear. Studying just how these antibiotics work looks like a promising avenue for developing new drugs that can wipe out persisters. For instance, last year it was shown that a TB antibiotic called pyrazinamide, which is somewhat effective against persisters, targets ribosomes, part of the cell’s protein-making machinery, and prevents them from restarting protein manufacture (Science, vol 333, p 1630).

Coates, however, has been trying to design antibiotics specifically to kill persisters rather than growing cells. His group had to develop new lab techniques to amass persister cells without awaking them, for instance, by starving them of nutrients, raising the temperature or lowering oxygen levels.

Hole punch

Coates’s group exposed its cultures to a wide range of compounds from commercially available drug libraries. The most effective of these – which they call HT61 – is extremely good at killing persistent cells (PLoS ONE, vol 5, e11818). “We start right from the beginning by targeting non-multiplying bacteria and that’s crucial,” says Coates.

The secret to this compound’s success is its ability to punch holes in the membrane and wall that surround every bacterium. Under the microscope, the cells look like burst balloons, with dark fluids leaking out of holes.

Because all bacteria have a membrane and wall, Coates hopes that this drug will be universally effective. So far, they have found it can kill Propionibacterium, which causes acne, Streptococcus and drug-resistant varieties of Staphylococcus. They are now testing it in a large trial against nose infections with Staphylococcus that is resistant to methicillin, otherwise known as the superbug MRSA.

Punching holes in bacteria seems a promising strategy. Last year O’Neill reported that natural plant chemicals called phenolic antioxidants can kill Staphylococcus biofilms, by perforating the bacterial membrane in a similar way. “They completely eradicate the biofilms,” he says. “You don’t see that with any approved antibiotics.”

Last year came news of another strategy: chemically nudging persisters out of their fully dormant state. The bacteria do not grow, but the cogs of their metabolism start to move very slightly, and the cells suck up substances from their surroundings, including antibiotics. This technique kills E. coli and Staphylococcus biofilms (Nature, vol 473, p 216).

Lewis is encouraged by the growing interest in this field, but he points out that the drug approval system doesn’t help matters. Except for with TB, regulators only want data on multiplying bacteria, so it is a waste of time, on the face of it, to test compounds against persisters. “There’s a big disincentive,” says Lewis.

Indeed, HT61 isn’t as effective against multiplying bacteria as other antibiotics on the market. If Coates had stuck to conventional tests, his drug would have been rejected.

But when it comes to doing things his own way, Coates can be pretty persistent himself. He has been studying persistor bacteria for about 15 years, and is also happy to see his favourite subject get attention from other scientists.

Targeting persisters will not eradicate the pressing problem of drug resistance, but it is opening up different ways of designing new antibiotics. And in times like these, every lead is precious.

“It’s early days, it’s high risk and we don’t know if it will work,” he says. “But we do need new approaches, and it is an exciting way forward.”

Ed Yong is a science writer based in London