The food chain: Antibiotics use in food animals

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AN UNWINNABLE WAR?

Wellcome Focus
Having been born in the antibiotic era – as was anyone born since penicillin entered the world stage in 1944–45 – I always assumed that bacterial infections were an irritation but manageable. As a child, I was fed antibiotics in a pink syrup to cure earache; as an adult, an IV drip poured antibiotics into my veins to defeat tonsillitis. Antibiotics have been taken for granted, always there, ready to counter infections of any part of the body.

Yet in the last few years, the doubts have started to creep in. Might bacteria be regaining the upper hand? Headlines trumpeting ‘MRSA is winning’ and ‘New lethal superbugs’ have become ever more frequent, as have reports of how bacteria have found ways to bypass, break down or just ignore our antibiotics. Microbiologists may wince at the term ‘superbug’, but it has become shorthand for infections of resistant bacteria caught in hospitals – which dominate the headlines – or in the community. Both are on the rise, while the flow of new drugs from the pharmaceutical industry has slowed to a trickle.

This Welcome Focus examines how science is responding to this challenge. Researchers are tackling bacteria from many different angles: how antibiotics work and the mechanisms bacteria use to resist the drugs; how we use of antibiotics, often excessive and uncontrolled, has driven the rise and spread of resistant strains; how these strains can be identified, tracked and their spread stopped; and how new drugs that can kill resistant infections are being developed. As the articles in this issue describe, progress is being made in all of these areas although there is still a lot to learn.

While Welcome Focus is looking at antibiotics and bacteria, resistance as a phenomenon is found in many other organisms. For example, widespread resistance in the malaria parasite has rendered the cheap and effective drug chloroquine almost useless. Resistance in the human immunodeficiency virus (HIV) threatens the usefulness of antiretrovirals. Attack any organism, it seems, and nature will almost always come up with a plan B to help survival. Humans have won notable battles in the war against infection – and antibiotics are still powerful weapons – but nature has evolution on its side, and the war against bacterial diseases is by no means over.

Dr Giles Newton, Science Editor

Introducing Welcome Focus...

This is the first issue of a new publication, Welcome Focus, which we intend to publish once a year. Our objective is to provide accessible, up-to-date and reliable guides to important and topical areas of medical science.

Research seems to progress at ever faster rates, and even specialists struggle to keep up with the deluge of information constantly being generated.

This information ends up in the primary literature, but that is a daunting starting point for anyone who does not already have a detailed understanding of an area. On the other hand, material for general audiences often focuses on healthcare delivery – but it can take a long time for the impact of new discoveries to be felt in medical practice.

With Welcome Focus, we hope to fill that gap. We hope to provide an overview of an area of medicine and key areas of research within it. Our belief is that this will be of value to a wide range of people, including: professional scientists reading outside their own area; healthcare workers of all descriptions; teachers looking for authoritative and topical resources for their students; other people with a professional interest in science and medicine; and, not least, members of the general public who are looking for a balanced and accessible insight into key issues in human health.

Our experience with Welcome News Supplements – such as last year’s, Talking Heads: Cognitive behavioural therapy comes of age – suggests that there is an appetite for this kind of publication. But if you feel there are other ways in which Welcome Focus could better meet your needs, do please let us know.

Introducing the Wellcome Trust...

The Wellcome Trust is an independent research-funding charity, established under the will of Sir Henry Wellcome in 1936. It is funded from a private endowment, which is managed with long-term stability and growth in mind. Its mission is to foster and promote research with the aim of improving human and animal health.

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Welcome Focus provides an overview of an area of medicine and key areas of research within it, through a mix of review articles, personal comment and research reports. It aims to provide scientists, healthcare workers, teachers, people with a professional interest in science and medicine, and interested members of the general public with a balanced and accessible insight into key issues in human health. It is available free (see inside back cover for ordering details).
**AN UNWINNABLE WAR?**

**Antibiotics and bacterial resistance**

Sixty years ago, the arrival of antibiotics seemed the answer to the bacterial diseases that have plagued humans through history. But are bacteria regaining the upper hand?

The last century saw a stunning swing in our fortunes against infectious bacteria: antibiotics, vaccination and clean water supplies slashed death rates, particularly in developed countries. With the rise of resistance to antibiotics in many species of bacterium, the pendulum seems to be swinging back.

This is not a new problem: the release of an antibiotic has usually been followed – sometimes quite soon after – by the appearance of bacteria oblivious to the new drug's effects. Indeed, even Sir Alexander Fleming pointed out in his 1945 Nobel Prize speech: “It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body.”

**Life with bacteria**

We live with bacteria all the time. They live on surfaces, on our skin, in our mouths, up our noses and in our intestines. By and large, we get along fine. One-third of the world’s population carries *Staphylococcus aureus* in the nose all the time and another third carries it now and again – and we have no idea it is there. Billions of bacteria, including *Escherichia coli*, live in our lower intestines. Indeed, this normal bacterial ‘flora’ is helpful, competing with and limiting the expansion of dangerous bacteria.

Antibiotics and vaccines also keep dangerous bacteria at bay. But humans have used antibiotics on an epic scale; in evolutionary terms, the selective pressures have been immense. Genetic changes that can help a bacterium resist a drug are rare, but bacteria reproduce so fast that they can disregard casualties. If a strain does pick up a mutation, or acquires a resistance mechanism from another, it will have a definite edge over its susceptible peers in the presence of the antibiotic. It is indeed survival of the fittest.

**Resistance at large**

That resistance should arise is no surprise: it is just nature’s way. What is surprising is how fast some strains of resistant bacteria can spread from person to person, from hospital to hospital and, aided by the increase in international travel, from country to country.

**Humans have used antibiotics on an epic scale**

Methicillin-resistant *S. aureus* (MRSA) has become synonymous with hospital-acquired infections, even though these bacteria account for only a proportion of the infections that affect about one in ten hospitalised patients. Hospitals are perfect for such ‘opportunist pathogens’: they are full of sick people with depressed immune systems; large amounts of antibiotics are dispensed; and catheters and surgery allow the bacteria to invade the body. Fortunately, few infections – whether of MRSA or other bacteria – are untreatable as we still have back-up drugs that work in most cases. Even so, patients have their stays prolonged and require additional diagnostic and therapeutic treatments; the additional costs are estimated to exceed £1 billion every year in the UK alone.

In the community, resistance is found in bacteria that cause pneumonia, earache, urinary tract infections, sexually transmitted infections and so on. Such resistant bacteria are a huge problem in developing countries, which still face an alarming death toll from bacterial infections: pneumonia and meningitis caused by *Streptococcus pneumoniae* are estimated to kill 1.6 million people every year and doctors are extremely worried by increasing rates of multidrug-resistant *Mycobacterium tuberculosis*. These countries may not be able to afford alternative drugs if cheap, first-line antibiotics become useless.

**Fighting back**

Twenty years ago, if an antibiotic became less useful because of resistance, there was always another drug coming along to solve the problem. This supply line has slowed markedly: it is now much harder to find new drugs (the ‘easy’ ones have been found) and many pharmaceutical companies have focused their efforts on more commercially rewarding markets. New potential targets for drugs have been identified from the sequencing of the genomes of bacterial pathogens – many of which have been deciphered – but it has taken much longer than expected to bring such drugs to market.

Improvements in hygiene, vaccines to boost our immune defences and, in the future, therapies based on bacteriophages (bacteria-killing viruses) can all have a role in slowing the spread of resistance. Even so, antibiotics remain our key drugs, and measures to preserve their utility have become a priority. Their use as growth promoters in food animals is being reduced, and prescribing for humans now aims to be more tailored and less frequent; in the UK, for example, total community prescribing has fallen by a quarter since the mid-1990s.

But at least part of the responsibility falls on us as consumers. Expecting instant cures, we pressurise GPs to prescribe antibiotics for inappropriate illnesses such as viral respiratory tract infections. As soon as we feel better, we hoard spare tablets in the medicine cabinet, sharing them with friends and family. Antibiotics are precious: we need to appreciate them more and be more thoughtful about their use.
Today, it is often forgotten that the challenge of antibiotic resistance was raised immediately the drugs were introduced. From the late 1940s, microbiologists and policy makers responded with attempts to restrict use through the prescription system and by the development of more robust drugs. Even then, the experts saw their measures as only partial solutions to a problem that was both medical and social. Yet in an age in which authority was increasingly distrusted, the warnings of pessimistic prophets were discounted by grateful patients and hurried practitioners alike. Only in the late 1990s was the emergence of resistant bacteria widely accepted as a global threat to be taken seriously.

Until the 1930s, there had been no chemical treatment available to fight bacterial infections in general. Prevention was the main means of protecting patients, and an obsession with the threat of germs and the moral responsibility to avoid infection were deeply instilled in Western cultures. At the same time, there were repeated hopes for a wonder drug. Louis Pasteur’s pupil Paul Vuillemin coined the term ‘antibiosis’ in 1889 to mean a process by which life could be used to destroy life. The word ‘antibiotic’ did not follow immediately, but the drug pyocyanase, a weakly effective antibiotic, was marketed from the late 19th century into the 1930s. Early in the 1920s, there was excitement about the potential of the newly identified phage viruses to attack bacteria on humanity’s behalf – remembered in Sinclair Lewis’s novel *Arrowsmith*.

The discovery of an antibacterial factor in the exudate of the *Penicillium* mould by Alexander Fleming at St Mary’s Hospital in 1928 was therefore not totally unexpected. Nor was it medically revolutionary. At first, it seemed impossible to extract the active compound intact from the yellow liquid produced by the mould, but two new developments in the late 1930s engendered more enthusiasm for the antibiotic approach to medicine. The sulfonamide drugs offered cures for a wide range of bacterial infections, and the Rockefeller Institute scientist Rene Dubos managed to extract a powerful antibiotic that he called tyrothricine from a soil mould, and later refined this to gramicidin and tyrocidine (although these could not be used internally).

Moreover, new chemical techniques and greater interest in natural chemicals such as proteins brought the problems of separating penicillin to the centre of pure science. It was scientific opportunity, not medical aspiration, that initially attracted the attention of the biochemist Ernst Chain and his boss, Howard Florey, at Oxford University to this challenge. Using the new technique of freeze-drying, Chain succeeded in separating penicillin and then showed that it did not harm mice.

With the intensification of war, medical implications replaced scientific curiosity as a driver for further work, and Florey put together a team of chemists, microbiologists and clinical scientists that tested penicillin on human patients during 1941. Yet it was clear that neither they nor British industry had the capacity to produce large quantities of penicillin, and the world centres of fermentation expertise were inaccessible in Nazi-occupied Europe. In desperation, Florey and his colleague Norman Heatley sought the help of colleagues in the USA. There, with much greater resources of fermentation experience and finance, and drawing on the advances made by the US Department of Agriculture research laboratory in Peoria, Illinois, a few companies such as Pfizer and Merck managed to develop mass-produced penicillin. By late 1943, the technology for mass production had been developed and by D-Day in June 1944, there was enough penicillin for all troops.
Meanwhile, at Rutgers University in New Brunswick, New Jersey, the achievement of Rene Dubos had encouraged studies of soil-derived actinomycetes, yielding a host of new drugs. In 1941, the professor of agricultural microbiology Selman Waksman coined the term “antibiotic.” He was emphatic that this type of antibacterial drug was necessarily derived from living organisms. Within the space of a few years, Waksman’s students separated actinomycin, neomycin, and, above all, streptomycin—the first drug to have a proven effect on tuberculosis.

Parke Davis extracted what came to be known as chloramphenicol from rotten vegetable remains sent from Venezuela.

Industry took up the challenge of finding active moulds, testing the chemicals they produced and mass-producing successful drugs. Parke Davis, a long-established firm that had been marginal to the penicillin enterprise, extracted what came to be known as chloramphenicol from rotten vegetable remains sent from Venezuela in 1946. At about the same time, Lederle Laboratories extracted aureomycin from a soil actinomycete. Pfizer, which had begun as a chemical company making drugs for retail-oriented pharmaceutical firms but then found that these firms were now manufacturing in their own right, began to seek drugs it could market itself. The first such product was terramycin, extracted from an actinomycete found in soil near its plant in New Jersey.

Chloramphenicol, aureomycin and terramycin differed from the first-generation antibiotics in that they attacked a wide range of bacteria, whereas their predecessors, penicillin and streptomycin, had been effective in general against only Gram-positive and Gram-negative bacteria respectively. The greatest of the early broad-spectrum antibiotics was developed by a Pfizer chemist, Lloyd H Conover, who realised that terramycin had a spectrum of antibiotics was developed by a Pfizer chemist, Lloyd H Conover, who realised that terramycin had a spectrum of action against only Gram-positive and Gram-negative bacteria whereas their predecessors, penicillin and streptomycin, had proved resistant to other antibiotics. Scientists at Beecham led the way in producing the core of penicillin (called beta-lactam) and then making new synthetic variants of penicillin. Some of these, such as ampicillin (1961) and the later amoxicillin, were broad-spectrum like tetracycline.

Before even ampicillin, however, in 1960 Beecham and Bristol brought out meticillin (methicillin), which could not be destroyed by the beta-lactamase enzyme produced by S. aureus. Almost immediately, cases of methicillin-resistant S. aureus (MRSA) were discovered, although for many years their number was low.

The new antibiotics had enormous consequences for both doctors and patients. Certain terrifying infectious diseases such as rheumatic fever, syphilis, pneumonia and tuberculosis became treatable and their disappearance seemed predestined.

The medicine chest

Famously, the life of the actress Elizabeth Taylor was rescued after she was treated for staph pneumonia during the shooting of the film Cleopatra. One response to the concern about resistance was the development of new antibiotics. Erythromycin and vancomycin were developed by Eli Lily in the 1950s and tended to be reserved for cases in which bacteria had proved resistant to other antibiotics. Scientists at Beecham led the way in producing the core of penicillin (called beta-lactam) and then making new synthetic variants of penicillin. Some of these, such as ampicillin (1961) and the later amoxicillin, were broad-spectrum like tetracycline.

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Meanwhile, the new drug seemed the solution to the threat of S. aureus. Famously, the life of the actress Elizabeth Taylor was rescued after she was treated for staph pneumonia during the shooting of the film Cleopatra. In part because of the new drug, the 80/81 epidemic faded away. Subsequently, methicillin was followed by more easily administered variants such as cloxacillin and flucloxacillin.

The medicine chest

Also in the early 1960s came the emergence of the cephalosporins (related to the penicillins) and another new family of antibacterial drugs (strictly not antibiotics, as they were totally synthetic) best known through the drug Cipro – later famously resorted to in the 2001 anthrax scare. However, this was also the end of the great period of antibiotic development.

In any case, the development of new antibiotics taken for short periods, often by patients with minor, self-limiting conditions, was not the principal concern of pharmaceutical companies more focused upon the challenges and opportunities of treating chronic conditions. Instead, the focus of the fight against resistance has been on managing the use of antibiotics and on preventing the selection of resistant strains.

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DRUGS IN PERIL

Antibiotics and resistance

How do antibiotics work? How do bacteria become resistant to antibiotics? If we reduced the amount of antibiotics we use, might resistant bacteria lose the upper hand? Lisa Melton explores the issues.

Antibiotics are the great warriors of modern medicine. These drugs have helped us to defeat infections that have threatened human life throughout history. But over the last decade, we have been losing ground at a dramatic pace. Infections considered curable – including tuberculosis, meningitis and gonorrhoea – are back to haunt us, and hospitals are infiltrated by bacteria that defy antibiotics. To quell the antibiotic backlash, scientists are striving to understand how antibiotics work and how bacteria resist their effects.

How antibiotics work

The best antibiotics are ‘magic bullets’ – a concept proposed by Paul Ehrlich in 1906 – that kill infectious organisms but do not harm the patient. By definition, antibiotic means ‘against life’, and most such compounds are natural products, chemosed from bacteria or fungi as chemical weapons to kill off other microbes.

The success of penicillin prompted an intensive search for similar compounds that could kill bacteria or stop them growing. Researchers sifted through the environment – soil, sea and sewer-infested waters – and came up trumps. Pharmaceutical companies also optimised the natural products by removing some chemical groups and adding others, to come up with new versions with enhanced benefits and minimal toxicity.

By targeting important biochemical reactions specific to bacteria, antibiotics tend to be harmless to people or animals. Some antimicrobials inhibit cell growth, giving the host’s immune defence a chance to wipe out the bacteria that remain. These drugs typically penetrate the microbe and interfere with the production of components needed to form new bacterial cells. For example, tetracycline binds to bacterial ribosomes, which make proteins, and in doing so hinders protein production.

Other drugs, such as the purely synthetic quinolones, stop DNA replication, and some, such as penicillin, upset the construction of the cell walls that protect bacteria from the outside world.

The bacterium’s rigid outer wall is critical to the cell’s survival – it imparts structure and support. Without it, the bacterial cell would explode owing to its own inner pressure and die. Because human and other mammalian cells lack such walls, penicillin and other related antibiotics (known as beta-lactams) are particularly safe.

The wall is made up mostly of peptidoglycan, a material that contains both peptides and sugars. To assemble it, peptide-sugar chains must be cross-linked together by an enzyme called transpeptidase, and it is the action of this enzyme that is blocked by penicillin. Penicillin does not really damage existing bacteria, but when the bacteria divide to make new cells, a new wall cannot cross-link properly. The wall becomes weak, like an ill-woven fabric, until it ruptures, killing the cell.

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Although the fungicide-derived cephalosporins are chemically quite different from the penicillins, they share the same mechanism of action: stopping transpeptidase. Cephalosporins are more expensive than penicillin and tend to be used if a person is allergic to penicillin.

There are, however, numerous semi-synthetic derivatives of penicillin, such as ampicillin (used to treat urinary tract infections), penicillin V and carbencillin (used for Pseudomonas infections). These compounds consist of the basic penicillin structure but have been modified in the laboratory to make them more stable, more effective against different bacteria, or more resistant to an enzyme called penicillinase. This is produced by most Staphylococcus aureus and some other bacteria, and it destroys penicillin.

But just as bacteria find new strategies to put antibiotics out of action, chemists have tricks too. By adding large bulky groups to the penicillin structure, it was possible to stop penicillinase from disabling the drug. As a result of this strategy, the semi-synthetic agent meticillin soon became the main clinical weapon against staphylococci that acquired resistance to penicillin.

Vancomycin, a glycopeptide, was first purified in 1956, but understanding how it works took three decades’ work. Gaining insight into its mechanisms was crucial because vancomycin has become the ‘antibiotic of last resort’ for the treatment of methicillin-resistant Staphylococcus aureus (MRSA). Chemically speaking, vancomycin is unusual: it is not related to other antimicrobials in use today. Although it also works by disrupting the bacterial cell wall, it latches onto the peptide strands directly, preventing them from growing into a polymer chain. Unfortunately, strains of vancomycin-resistant bacteria have been reported (see below).

Bacteria bite back

Shortly after penicillin came into widespread use, a strain of resistant S. aureus emerged. But pharmaceutical companies developed a string of other drugs, including new versions of penicillin, that could control these strains. Clinicians were confident that, should a bacterium develop resistance to a particular antibiotic, throwing another drug at it would work.

So how do bacteria pull off this survival trick? Natural selection is the key. Bacteria multiply rapidly, going through many cell divisions a day, and some cells may acquire random genetic mutations. If the genetic change happens to affect the target molecule of an antibiotic, for example, those bacteria could become
resistant. When confronted with an antibiotic, the mutants will have the competitive advantage. Natural selection will ensure that mutants thrive while the rest are wiped out. That's the rub: antibiotics. One resistance mechanism alters the final amino acid D-alanine in the peptidoglycan chains that form the wall. The antibiotic normally needs two D-alanines to latch on to; if it can no longer do this, the peptide chains are free to link up tightly again to form the wall. Another mechanism is to substitute D-alanine for a much larger amino acid, a tactic that keeps vancomycin from binding.

Vancomycin was in use for 30 years before resistance first emerged in gut flora. These bacteria, known as enterococci, are normally harmless unless they invade other parts of the body. But a chill ran through the clinical world when, in 1997, scientists discovered that an MRSA strain had picked up vancomycin-resistance genes. These resistant bugs bypass vancomycin interference by thickening the peptidoglycan mesh in the bacterial cell wall without resorting to cross-linking. Vancomycin’s meddling makes no difference because thickness has replaced interweaving.

Resistance and fitness

If bacteria can gain resistance to antibiotics, can they lose it too? Resistance does not necessarily come for free: a bacterial protein may be impervious to antibiotic attack but less efficient at its job; bacteria hosting plasmids full of resistance genes may take longer to replicate their DNA; and producing pumps to expel antibiotics from the cell or extra proteins to thicken up the cell wall, may drain energy that would otherwise be used for the fastest growth and reproduction possible.

If the antibiotics were removed from use, might the resistant strains lose their edge?

In the presence of antibiotics, resistant bacteria have an undoubted advantage: their susceptible peers are killed off and slow growth is an acceptable price to pay. But if the antibiotics were removed from use, might the resistant strains lose their edge, and be outcompeted by leaner, faster-growing susceptible strains? A key issue is ‘fitness’, a measure of how well a bacterial strain can infect a host, persist and proliferate, and be transmitted to a new host. Although several resistance mechanisms have been shown to impair fitness, others seem to be gratuitous, affecting growth little, if at all.

Even if a resistance mechanism does cause a bacterial strain to slow down, it may become fitter if it gains other mutations – compensating mutations – that ameliorate the costs of resistance. For example, a resistance gene is carried on transferable DNA such as a plasmid, it may get into a strain that can host it more easily. Fit strains with potent resistance mechanisms, such as multidrug-resistant Acinetobacter baumanii that plague intensive care units and the epidemic MRSA strains that have swept into hospitals, are real headaches for modern healthcare.

Dr Lisa Melton is a freelance writer based in London, UK.
**MRSA MYSTERY**  
A patient’s experience of MRSA

When Alison went to a leading London hospital for an operation, she expected to be home in three weeks. But after contracting MRSA, it was a different story.

"I was due to have an operation at a leading London hospital. Before I went in, I got a letter from the hospital saying it was high up in the anti-MRSA league tables. I thought: ‘Oh good, that’s one thing I don’t need to worry about’.

I’d had an operation four years previously, and on that occasion, they swabbed me in hospital on my first day to check I wasn’t bringing any infection in. So I was surprised that the procedure wasn’t repeated this time round. They talked to me about the operation, but didn’t take any swabs.

> I was delirious during my time in the High Dependency Unit – 33 days in all – and remember very little of it. It was like a dream. >

After the second operation (there were two stages), I was suddenly moved to an isolated cubicle in the High Dependency Unit (HDU), where full isolation procedures were followed (staff and visitors had to wear sterilised gloves and gowns in the cubicle, and they had to wash their hands in alcohol gel before coming in). Here, they told me my swab had shown I had MRSA but said I had nothing to worry about because it was isolated in the bladder.

> ‘MRSA’ was highlighted in big red letters. >

When I improved, I was taken from HDU to another isolation ward. When I asked why I wasn’t going back to the open ward, the nurse hesitated slightly, then said I would find it easier to rest in a single cubicle. Again in this ward, they followed isolation procedures. The hygiene was good, but they talked about the MRSA very casually. They said it comes and goes, and that I didn’t need to worry about it because I wasn’t symptomatic.

Once I could speak and think more clearly, I asked a doctor if the MRSA had got into my lungs. He looked thrown, then said he thought he remembered traces in the pleural fluid but he could be wrong. Most of the nurses didn’t know. Eventually I found one who confirmed I had had MRSA in my lungs.

All in all I was in hospital for nearly ten weeks – although my operations and recovery period should only have taken three. When I left they gave me a letter for the district nurse. It said I’d had MRSA (‘MRSA’ was highlighted in big red letters) – not just in the pleural fluids, but in the nose as well. They hadn’t said that in hospital.

> ‘MRSA’ was highlighted in big red letters. >

Overall, although care and prevention was excellent, communication was poor. I have no knowledge of the course of the MRSA, how or when I got it, where or when the tests were taken, or when new negative or positive results came in. No one was ever open or specific about it; it wasn’t straightforward. Yet they were always very clear and precise about my operations."

"We do not yet know how many species of bacterium exist – science has identified several thousand species, and the total number may well run into the millions – but it is already clear that bacteria are remarkably diverse. Each species has its own characteristics, and different strains within just one pathogenic species can have quite different abilities to cause disease or variable sensitivity to antibiotics.

With the prevalence of antibiotic-resistant bacteria on the up, and their dissemination worldwide, it has become increasingly important to be able to recognise and distinguish different strains of the bacteria that endanger humans. Using sophisticated methods such as multilocus sequence typing (MLST), the spread of particularly virulent or resistant strains can be tracked, their origins traced and the routes of disease transmission understood.

**Profiling bacteria**

MLST has rapidly become the ‘gold standard’ for characterising strains of important pathogens such as Streptococcus pneumoniae, Staphylococcus aureus and Enterococcus faecium. In this approach, the DNA sequences of fragments of seven genes are obtained from bacterial isolates, and each of the different sequences (alleles) at each of the seven genes is assigned a different number. (The technique examines metabolic genes, which keep the bacterium running and, unlike genes involved in colonising a human or in resisting antibiotics, tend to evolve quite slowly.)

A bacterial isolate can be characterised unambiguously by a string of numbers – the allelic profile – that corresponds to the DNA sequences at the seven loci. In MLST, isolates with the same sequences at all seven loci are considered to be the same strain and are assigned a ‘sequence type’.

Bacteria are the most numerous and diverse organisms on the planet. Brian Spratt explores how the latest techniques are tracking strains resistant to antibiotics, and shedding light on their origins and spread.

**Tracking resistant strains of bacteria**

> continued on page 14

International MLST databases of this type have been developed for a number of major bacterial pathogens, and these continue to grow as those studying the pathogen add data on their isolates to the central database.

Brian Spratt explores how the latest techniques are tracking strains resistant to antibiotics, and shedding light on their origins and spread.
The origins of resistance
A close study of bacterial DNA can also shed light on the origins of resistant strains. New strains emerge and then start to accumulate genetic variation, resulting in the presence within a bacterial population of many sets of related strains (sometimes called clonal complexes), each typically including the ancestral genotype and a diverse set of minor variants.

MLST has been extremely useful for clarifying how different countries’ antibiotic-resistant strains relate to one another and for establishing a common nomenclature. Using a central database at www.mlst.net, a microbiologist can determine whether a major antibiotic-resistant strain in one country is new (perhaps having emerged there), or has already been reported from other countries (suggesting that it has been imported). Important new strains of antibiotic-resistant *Streptococcus pneumoniae* are now defined by MLST, and the previously chaotic naming of strains of methicillin-resistant *Staphylococcus aureus* (MRSA) has been rationalised using MLST and the molecular features of the genes that encode methicillin resistance.

Not all bacteria are amenable to MLST, having too little sequence variation in their metabolic genes for the technique to discriminate between strains. One such example is *Neisseria gonorrhoeae*, and so a technique termed NG-MAST, which examines the variation in two rapidly evolving genes, has been used to understand the importation and spread of gonorrhoea. In the last few years, the prevalence of *N. gonorrhoeae* resistant to ciprofloxacin, the standard treatment, has increased so much so that this drug is no longer considered to be the antibiotic of choice for uncomplicated gonorrhoea.

The resistant strains emerged in the Far East, and NG-MAST has shown that those in the UK were at first unique strains, associated with male heterosexuals with a history of sex tourism. Now, however, large clusters of people share the same strain. People within these large clusters report high rates of partner exchange, suggesting that the increased prevalence of resistance is largely due to the introduction and endemic spread of ciprofloxacin-resistant strains within such high-risk groups.

The most prevalent antibiotic-susceptible *S. aureus* strains in hospitals have become methicillin-resistant repeatedly.

Computer software that models such family trees and maps the presence of resistance genes shows that while the resistant strains of a pathogen often spread and gradually diversify, the resistant genes sometimes move into new strains. Both of these mechanisms are evident in the emergence of penicillin-resistant *S. pneumoniae* and MRSA. Furthermore, individual strains may acquire resistance repeatedly, and this is also seen in MRSA: the most prevalent antibiotic-susceptible *S. aureus* strains in hospitals have become methicillin-resistant repeatedly, acquiring different forms of the methicillin resistance genes.

Resistance tends to emerge in those strains that are most exposed to antibiotics. In community-acquired *S. pneumoniae*, penicillin resistance has emerged largely in those strains that tend to colonise the nasopharynx of children, while in *S. aureus* it is the prevalent antibiotic-susceptible strains within hospitals that have gained resistance to methicillin. These hospital strains may already be particularly well adapted to colonisation and transmission among patients and healthcare workers in hospitals; acquiring resistance gives them a further edge, and the new strains come to dominate wherever antibiotics are widely used. The new strains are likely to be the ones in which resistance to further classes of antibiotic will appear, leading to the build-up of successful multi-resistant strains in hospitals.

This appears to have occurred in *S. aureus*, where methicillin resistance (and subsequently multiple antibiotic resistance) has arisen within the most widespread susceptible strains, and decreased susceptibility to vancomycin has also recently appeared in these successful MRSA strains.

Natural selection reduces or eliminates any ‘cost of resistance’

It was once the view that acquisition of resistance resulted in bacteria that were somewhat crippled. If antibiotic usage were reduced, the theory went, the resistant strains would be outcompeted by fitter antibiotic-susceptible strains and thus eliminated. It is now realised that natural selection reduces or eliminates any ‘cost of resistance’ and it appears that well-established antibiotic-resistant strains have only slight reductions in fitness. The most problematic strains have also gained resistance to many of the available classes of antibiotic; limiting the use of only one antibiotic class is unlikely to have any significant impact on the prevalence of such strains.

The very high rates of MRSA and some other pathogens in hospitals could arguably be due to very high levels of selection for resistant strains, resulting from frequent antibiotic usage. However, the increasing recovery of MRSA strains in the community – or the rapid international spread and high prevalence in many countries of multi-resistant *S. pneumoniae* among children – makes it hard to argue that antibiotic-resistant strains are substantially disadvantaged.

Reversing the high prevalence of antibiotic strains in hospitals or the community is unlikely to be easy.
THE RISE OF RESISTANCE

Resistance in the UK

Dr David Livermore, Director of the Antibiotic Resistance Monitoring and Reference Laboratory, sees bacteria from hospitals across the UK. And not just any bacteria: these are the strains most resistant to antibiotics.

What’s the role of your laboratory?

We’re the UK’s national reference laboratory. When hospitals find bacteria with unusual or unknown resistances, they can send them to us for the resistance to be confirmed, for treatment advice, and for the public health importance to be gauged. We also do research on the mechanisms of resistance, and work together with epidemiologist colleagues on the surveillance of antibiotic resistance.

Reports of resistance to vancomycin – the ‘drug of last resort’ in methicillin-resistant Staphylococcus aureus (MRSA) – imply that MRSA infections will soon be untreatable. Is this the case?

MRSA is essentially an infection-control problem. In the UK, two ‘epidemic’ MRSA strains, EMRSA-15 and -16, have become dominant and have spread widely. They are tenacious, but don’t have a huge spread of resistance to antibiotics. Contrary to what is often said, we do still have several antibiotics, not just vancomycin, that are active against MRSA, particularly against these two strains.

There have been a few cases of vancomycin resistance or intermediate resistance in MRSA, but there are half a dozen or so new antibiotics coming along that are active against MRSA. It remains to be proved, though, whether these are better than vancomycin.

Is resistance to vancomycin common in other bacteria?

Resistance to vancomycin is much more prevalent in the enterococci (the gut-dwelling Enterococcus faecium and E. faecalis, which are also opportunistic pathogens) than in staphylococci, although the statistics are a little complex... E. faecalis causes 90 per cent of enterococcal infections, but its rate of vancomycin resistance is only 2–3 per cent. For E. faecium, the rate of vancomycin resistance is 20–25 per cent but it only accounts for only 10 per cent of enterococcal infections, mostly in specialist units such as renal units, transplant units and haematology units. It’s not like MRSA, which causes infections widely in hospitals.

Resistance in Streptococcus pneumoniae is quite high in some parts of the world. How does the UK compare?

In the UK, we don’t have a big resistance problem in S. pneumoniae – 3–5 per cent have resistance to penicillin, and this is usually low-level resistance which can be overcome by increased doses, except in meningitis. Rather more, 12–15 per cent, are resistant to macrolides. So, as long as the patient is not allergic, you usually can give them a high dose of penicillin. If they are penicillin-allergic, then the rate of macrolide resistance is perhaps pushing us to use alternatives, such as fluoroquinolones. If you go to the USA, southern Europe or South-east Asia, there are much bigger problems with penicillin resistance in pneumococci, probably as a result of more community prescribing and selection pressure.

Do bacteria that cause food poisoning show significant resistance to antibiotics?

By and large you wouldn’t treat a Salmonella gastro-intestinal infection with antibiotics: you just let it burn itself out. But resistance has been going up, both to quinolones and cephalosporins, so when you do have to treat someone – for example, a neonate with a salmonella meningitis or someone with typhoid fever – it is getting harder. Campylobacter shows increasing fluoroquinolone resistance, but alternative drugs are macrolides, where there still isn’t much resistance. Escherichia coli is more complex, because different strains cause different problems. Many strains have harmless in the gut, but some – such as E. coli 0157 – can cause dangerous gastrointestinal disease. By and large, you wouldn’t treat infections due to these strains with antibiotics, but nor are they very resistant.

Other E. coli strains are the major causes of urinary tract infections, and most cases of E. coli blood poisoning reflect overlap from these infections. Here there are growing resistance problems. More than half the isolates are resistant to ampicillin and about a fifth are resistant to trimethoprim. Worse, there is fast-emerging resistance to the cephalosporins and quinolones such as ciprofloxacin. Until recently, these were very good treatments but now we’re being pushed towards using carbapenems for severe infections with E. coli. These are powerful, injectible antibiotics that we’ve previously tended to keep in reserve.

Which bacteria are cause for most concern?

We are in most trouble with some of the Gram-negative pathogens that mostly affect patients in intensive care, especially Acinetobacter baumannii. Over the years, it has become progressively more resistant to all antibiotics and there are now strains circulating that have resistance to carbapenems, which were the last good line of defence. Some intensive care units in London are now having to use polymyxin, a rather toxic old antibiotic that had largely been abandoned for systemic use. And there is little in the way of new antibiotics becoming available that are active against Acinetobacter. There is one new tetracycline analogue but, beyond that, nothing.

So, treatment options are narrowing against Gram-negative bacteria. These include both E. coli, a very common pathogen, and Acinetobacter, which is less frequent, but is important in intensive care medicine. And against some of these less common intensive care pathogens, we’re being forced to use some not very good, and rather unpleasant old antibiotics.

Dr David Livermore is Director of the Health Protection Agency’s Antibiotic Resistance Monitoring and Reference Laboratory, Colindale, London, UK.

Dr Livermore's laboratory, the Antibiotic Resistance Monitoring and Reference Laboratory, monitors and researches antibiotic resistance in bacteria. This is crucial for understanding how bacteria evolve resistance to antibiotics and for guiding clinical decisions. Resistance to antibiotics is a global issue, with MRSA, E. coli, and Acinetobacter being of particular concern.

MRSA, for example, has become resistant to vancomycin, one of the last resorts for treating MRSA infections. This is due to resistance genes spreading among bacteria through horizontal gene transfer. Dr Livermore's laboratory plays a key role in tracking the spread of these resistance genes and in developing strategies to combat antibiotic resistance.

The laboratory also focuses on other resistant bacteria such as E. coli and Acinetobacter, which are important causes of urinary tract infections and infections in intensive care units, respectively. They are increasingly resistant to antibiotics, making treatment challenging.

Understanding the mechanisms of resistance and the spread of resistant strains is essential for developing effective treatment strategies and for informing guidelines on the use of antibiotics. Dr Livermore's laboratory contributes to these efforts, ensuring that healthcare professionals have the most up-to-date information to manage resistant bacterial infections.
“MRSA IS NOT A SUPERBUG”
A doctor’s view of MRSA

Dr Graeme Wilson is at Newham Hospital, London, UK.

The spread of resistant bacteria in hospitals has become a major public health and political issue. But Mark Wilcox points out that we do not necessarily know the most effective ways to control this spread.

The spread of resistant bacteria such as MRSA is preventable, as countries such as Sweden, Denmark and The Netherlands have shown.

Control measures also need to be practical, given available resources. For example, new methods to detect MRSA earlier, including new tests that cut detection times from two days to two hours, may not actually reduce infection risk unless effective treatments or preventative approaches (such as the capacity to segregate patients) can be implemented. Much has been written about hospital cleanliness and the risk of hospital infection, notably the spread of MRSA, but the lack of data to substantiate a link between these is stark.

We also do not know what happens when we stop using compromised antibiotics. There is evidence that some types of resistance will persist, while other types are reversible (if, for example, the prescribing of a specific antibiotic is restricted). But, perhaps

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surprisingly, there is little robust information on how best to optimise and regulate antibiotic prescribing in hospitals. Many data have been collected about the extent of antibiotic resistance, but rarely is it also determined how commonly antimicrobials are used. So while we know that antibiotic prescribing by GPs has declined recently, comparable information on antibiotic prescribing in hospitals – at the national level – is not freely available. We also need to know more about the long-term effectiveness of restricting antibiotic prescribing, especially where one drug is substituted for another, or where antibiotics are rotated in a hospital or unit (to reduce the time that any one antibiotic has to induce resistance). While antibiotic rotation is attractive in theory, it has yet to be proved both sustainable and efficacious at reducing levels of resistance.

More uncertainty

We need to know a lot more about the genes that allow bacteria to resist antibiotics. For example, there is uncertainty about the potential for genes to jump from bacteria carried often harmlessly by humans or animals (such as the normal flora in the gut) to pathogenic bacteria. An important area for progress is predicting more rapidly when infections are likely to respond to which antibiotics. This need extends beyond knowing whether or not a bacterium has a certain resistance gene; we also need to know what makes it so effective (its virulence determinants) and accessory genes that may control its response to antibiotics. For example, there is increasing evidence that bacterial regulator genes may affect the likelihood of vancomycin therapy succeeding against MRSA infection.

It is important to recognise that antibiotic resistance does not respect boundaries between hospitals and the community; the interplay between these pools of resistance genes is not well understood. Recently, virulent strains of so-called ‘community-associated’ MRSA (to distinguish them from healthcare-associated strains) have emerged. Such strains have different resistance genes and have spread in a number of groups – such as children, sports teams and prison inmates – previously not considered to be at increased risk of MRSA infection.

Another recently reported phenomenon is the emergence of Gram-negative bacteria that can produce enzymes (beta-lactamases) rendering commonly used antibiotics – penicillins and cephalosporins – ineffective. For example, resistance to third-generation (extended-spectrum) cephalosporins in *E. coli* – a common cause of urinary tract infections and sometimes serious invasive infection such as septicaemia – has remained below 10 per cent in most European countries. However, there are sporadic reports of more prevalent resistance emerging in some UK hospital and community settings and in some eastern European countries. There has also been a consistent and marked rise in fluoroquinolone-resistant Gram-negative bacteria and, worryingly, clinicians are now faced with the emergence of Gram-negative bacteria resistant to all approved antibiotics (such as Acinetobacter species; see page 17).

As the length of hospital stays decreases, and as in general community and secondary care become more closely interwoven, our surveillance for and understanding of the epidemiology of antibiotic resistance emergence will have to become a lot more sophisticated.

Concluding thoughts

We must not overplay the issue of antimicrobial resistance, and patients can be reassured that the vast majority of infections remain treatable. However, we must never assume that we have finally beaten the microbes, no matter what new drug is discovered; rather, we must try to remain one step ahead. Antibiotic prescribing must be used judiciously. Vaccination against infectious diseases – which has transformed medicine – has the potential to make significant inroads into the control of healthcare-associated pathogens. For example, a vaccine against *S. aureus* has given promising results in clinical trials.

Last but not least, hospitals need to prioritise good infection-control practice – resources spent on waiting lists targets need to be redirected or preferably increased. Hospitals of the future need a greater supply of single-room accommodation to help contain the spread of antimicrobial resistance.

Mark Wilcox is Professor of Medical Microbiology, Clinical Director of Microbiology, and Director of Infection Prevention and Control at Leeds Teaching Hospitals and the University of Leeds, UK.
Methicillin-resistant Staphylococcus aureus

Why have two strains of methicillin-resistant Staphylococcus aureus (MRSA) infiltrated UK hospitals? How do they relate to MRSA elsewhere in the world and to ‘community-acquired’ strains? Mark Enright is tracking the answers.

At the beginning of the 1990s, UK hospitals started to identify two new strains of methicillin-resistant Staphylococcus aureus (MRSA). Such resistance was nothing new: the first case had been found in 1961, just one year after methicillin had been introduced. But these new strains spread with such astounding efficiency that, a decade later, most UK hospitals have been infiltrated.

“Modern medicine gives these organisms easy entry to the body,” says Mark Enright. “More invasive surgery is being performed, more catheters are being put into people’s bloodstream, and an older, sicker, more vulnerable population is being treated. Someone coming along having treated a patient with MRSA, their more vulnerable population is being treated. Someone to be particularly well suited to life in hospitals. They get into the body and invasive disease results.”

The two strains, epidemic MRSA-15 and -16, seem to be particularly well suited to life in hospitals. These would be worrying developments.”

Not all cases of MRSA arise in hospitals. Community-acquired strains: “There was an idea that they were feral hospital strains. ‘There was an idea that they were feral hospital strains. But these new strains spread with such astounding efficiency that, a decade later, most UK hospitals have been infiltrated. “Modern medicine gives these organisms easy entry to the body,” says Mark Enright. “More invasive surgery is being performed, more catheters are being put into people’s bloodstream, and an older, sicker, more vulnerable population is being treated. Someone coming along having treated a patient with MRSA, their more vulnerable population is being treated. Someone to be particularly well suited to life in hospitals. They get into the body and invasive disease results.”

Community strains could become more like the hospital strains and become harder to treat. Or hospital strains could become more dangerous if they acquire toxin genes

Using MLST, Dr Enright has found that community-acquired strains are very different from hospital strains: “There was an idea that they were feral hospital strains. Instead, some community isolates turn out to be descended from a penicillin-resistant strain of S. aureus that appeared in the 1950s (see page 6). The type 80/81 strain was notable in its day for being unusually transmissible and virulent, and was probably the most frequent cause of severe staphylococcal disease in the mid-to-late 1950s. This strain was supposed to have been vanquished by methicillin in the 1960s. Not so, Dr Enright and colleagues have found: the strain has persisted and is now re-emerging in the community, this time resistant to methicillin.

Tracking MRSA

Using multilocus sequence typing (MLST; see page 13) to ‘DNA profile’ different strains of S. aureus, Dr Enright is seeing where they are descended from and how they fit into families. “Data from more than 1000 isolates of S. aureus from about 15 different countries in the database,” he says. “You can see where a strain has spread in the world, if it has caused disease in children or adults, and if it is in hospitals or communities.”

Using these data, Dr Enright has found that today’s hospital MRSA strains descend from five original strains that independently gained methicillin resistance. “MLST shows the global epidemiology of different strains and their evolution in the long term,” he says. “We can plot the relationships between the strains and see how descendants emerge.” These strains have also spread worldwide. “Whatever you find in the USA you’ll find in the UK, albeit at a low frequency at first. Even when we tested strains from Cuba – from local hospitals that aren’t accessed by tourists – we found that hospital strains are similar to those in other countries.”

Tracking the opportunist

Using MLST, Dr Enright has found that community-acquired strains are very different from hospital strains: “There was an idea that they were feral hospital strains. Instead, some community isolates turn out to be descended from a penicillin-resistant strain of S. aureus that appeared in the 1950s (see page 6). The type 80/81 strain was notable in its day for being unusually transmissible and virulent, and was probably the most frequent cause of severe staphylococcal disease in the mid-to-late 1950s. This strain was supposed to have been vanquished by methicillin in the 1960s. Not so, Dr Enright and colleagues have found: the strain has persisted and is now re-emerging in the community, this time resistant to methicillin.

Community strains could become more like the hospital strains and become harder to treat. Or hospital strains could become more dangerous if they acquire toxin genes

If the evolution of MRSA continues apace, the concepts of hospital and community MRSA strains may become blurred. As Dr Enright points out: “Community strains could become more like the hospital strains in terms of antibiotic resistance and become harder to treat. Or hospital strains could become more dangerous if they acquire toxin genes, and could cause serious disease in younger, healthier people. You might not need catheters to have MRSA in hospital. These would be worrying developments.”

Dr Mark Enright is at the Department of Infectious Disease Epidemiology, Imperial College London, UK, and holds a Wellcome Trust project grant.

MRSA: GENOMIC CONFESSIONS

Within the genome sequence of Staphylococcus aureus are the genes that make it tick, that enable it to colonise a human and perhaps cause disease, and that, in some strains, help it to resist antibiotics. By Matt Holden.

These genes are not necessarily ‘home-grown’: with the genomes of several strains complete, it is becoming clear that S. aureus is adept at acquiring and transferring mobile pieces of DNA containing resistance genes.

The genomes of two S. aureus strains were completed at the Wellcome Trust Sanger Institute in 2004. MRSA252 is a hospital-acquired representative of the epidemic methicillin-resistant EMRSA-16 clone responsible for half of the MRSA infections in UK hospitals, while MSSA476 is an invasive community-acquired methicillin-susceptible strain (MSSA).

Approximately 80 per cent of the S. aureus genome is common to all the strains. Here lie many of the genes that keep the bacterium running and help it to colonise or infect humans. The regions that differ are of variable size, are scattered throughout the genome and contain genes found in only one, or a few, of the strains. These regions often contain genetic elements that can be transferred between strains. One such element in MRSA252 – termed the staphylococcal chromosome cassette – carries the methicillin resistance gene as well as genes that confer resistance to erythromycin, spectinomycin, kanamycin and tetracycline. The chromosome of MRSA252 also contains another two mobile elements carrying a beta-lactamase (an enzyme that destroys penicillins), and erythromycin and spectinomycin resistance genes. In fact, all but one of the antibiotic resistance genes in MRSA252 are encoded on mobile genetic elements.

The methicillin-sensitive strain MSSA476 is resistant to fewer antibiotics, but has also gained a selection of mobile DNA elements. With the exception of genes in two regions in the genome, all of the genes in MSSA252 are found in a community-acquired strain called MW2 (whose genome has also been sequenced), closely related to MSSA252 but methicillin-resistant. Since these strains diverged from a recent common ancestor, it appears that five horizontal exchange events have occurred. Strikingly, the two strains have staphylococcal chromosome cassette elements carrying different antibiotic resistance genes at the same location on their chromosomes. In the case of MSSA476, it contains a novel type of cassette with a gene that confers fusidic acid resistance.

This comparison illustrates the important role that mobile elements have in gene exchange within lineages, and the potential for rapid emergence of new drug-resistant strains of this pathogen.

Dr Matt Holden is at the Wellcome Trust Sanger Institute, UK.
THE FOOD CHAIN
Antibiotic use in animals

Antibiotics keep food animals healthy. But their use is being curtailed amid growing evidence that resistant bacteria can spread from animals to humans, as Henrik Wegener describes.

It is estimated that more than half of all antibiotics produced worldwide are used in animals. Although some are for our companion animals, most are used on food animals to treat infections that can devastate herds, to prevent infections or to boost production. But worries have arisen that, in the long term, human health could be severely threatened. All of the different types of antibiotic that are used in animals are also used in humans, and mounting evidence of resistant bacteria developing in animals and infecting humans – or passing their resistance genes onto human-infesting bacteria – is leading governments to cut back animal antibiotic use.

Any use of antibiotics can lead to the development of resistance. In general, high concentrations used for a short time, such as when treating sick animals, are less likely to lead to resistance than low dosages for a long time, such as in growth promotion. And resistance has indeed arisen: before the growth promoter avoparcin was banned in Denmark in 1995, 80 per cent of Danish broiler chickens had enterococci resistant to the human antibiotic vancomycin, a close relative of avoparcin.

But does the use of antibiotics in animals actually affect human health? While we do not know how much of the resistance problem seen in humans is attributable to animal use of antibiotics, clear examples have been seen with Salmonella and Campylobacter. These bacteria cause an estimated 200 million infections, primarily diarrhoea, worldwide each year; most of these infections originate from animals. In recent years, a multi-resistant form of Salmonella (S. typhimurium DT104) has spread in animals, foods and, subsequently, in humans. The massive spread of this strain around the world has been attributed in part to its advantage in environments with frequent antibiotic use. Studies have indicated that this multi-resistant strain may cause more severe infections in humans.

Another worrying trend has been observed recently. Salmonella and Campylobacter strains resistant to quinolones have emerged in animals and spread to humans. These drugs are used routinely in humans for the treatment of acute and severe diarrhoea, but recent studies have shown that infections with quinolone-resistant Salmonella and Campylobacter tend to be more severe and more often fatal compared with infections with sensitive strains.

Animals and humans
As they are kept in large, high-density groups and often raised to slaughter weight before they reach physical maturity, it is not surprising that food animals can develop and spread infectious diseases easily. In response, farmers have used antibiotics liberally to keep their stocks healthy. Entire groups of animals are treated as soon as clinical symptoms appear in one animal, and many groups of animals are treated before symptoms appear. But the most controversial use of antibiotics – and the most common use – is in ‘growth promotion’. By some mechanism, as yet unclear, the addition of low doses of antibiotics to animal feed can enhance growth rates and increase feed efficiency. Such antibiotics can be used throughout an animal’s life.

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Cutting back
Given reports that antibiotic growth promoters had created a huge reservoir of resistant bacteria in animals, and documented examples of the transmission of these resistant bacteria from animals to humans, governments have taken action. Denmark has been a leading proponent of such moves. In 1995, it stopped the use of avoparcin as a growth promoter, and in just three years the prevalence of vancomycin-resistant enterococci in Danish poultry fell from over 80 per cent to under 5 per cent. In 2000, the use of any antibiotic growth promoters was stopped and, in 2003, strict regulations were imposed on the use of quinolones in animals. Danish vets can now only prescribe quinolones if they can document that no other antibiotics would be effective. This has markedly reduced the use of quinolones in animals and limited the occurrence of resistance.

Europe-wide measures have also been taken. The use of avoparcin in animals was banned in 1997, and all uses of antimicrobials for growth promotion will be banned from 2006. The impact on farmers may be smaller than feared: five years after Denmark stopped the use of antibiotic growth promoters, only minor negative effects have been seen (some pig herds have seen diarrhoeal problems in weaned piglets). There has been a rapid and major decline in the occurrence of bacterial resistance to these antibiotics in animals and food, and Danish food animal production has continued to increase. With modern farming practices, it may well be that antibiotic growth promoters are simply not as necessary as they were in the past.

Dr Henrik C Wegener is at the Danish Institute for Food and Veterinary Research.
Antibiotic resistance in community bacterial infections

Angela Brueggemann discusses the pneumococcus, while Penny Bailey reports on research in Wales on the correlation between prescribing patterns and antibiotic resistance.

Hospital-acquired bacterial infections may dominate the headlines, but most infections occur in the community. Indeed, 80 per cent of antibiotic prescribing takes place in the community—in local practices, daycare centres and long-term care facilities such as nursing homes and rehabilitation centres. General practitioners (GPs) have to tackle a wide range of bacteria, including: Streptococcus pneumoniae, Staphylococcus pyogenes, which most often causes ‘strep throat’, a mild sore throat; Neisseria meningitidis, an important cause of bacterial meningitis; Campylobacter and Salmonella, which cause bacterial gastroenteritis; and Escherichia coli, responsible for most urinary tract infections—more than 80 per cent of cases of acute uncomplicated cystitis in young women.

The pneumococcus

Community-acquired infection with Streptococcus pneumoniae (the ‘pneumococcus’) is the leading bacterial cause of human illness and death worldwide. First identified in 1881 by Sternberg and Pasteur, the bacterium causes life-threatening diseases such as pneumonia, blood-stream infection and meningitis, as well as sinusitis and acute earache (otitis media) — the most frequent illness for which antibiotics are prescribed for children in industrialised countries.

Antimicrobial resistance among S. pneumoniae was first recognised in 1917, when patients with pneumococcal infections were treated with ethylhydrocupreine (optochin) and resistance developed while the patients were in therapy. Penicillin became the treatment of choice from the 1940s, and then restarted in the late 1960s. A vaccine suitable for adults was developed in the 1970s, but recent efforts have focused on a newer vaccine, suitable for children, which was licensed in 2000. In addition to a dramatic reduction in the incidence of invasive pneumococcal disease, it was reported in March 2005 that the use of this vaccine in US children was associated with a reduction in macrolide resistance in S. pneumoniae, an encouraging result.

Dr Angela Brueggemann is at the Department of Microbiology and Infectious Disease, and the Department of Public Health and Primary Care, University of Oxford, UK.

During the 1980s and 1990s, resistance among S. pneumoniae became a problem of global significance, affecting many countries. For example, in the USA, penicillin resistance rose from less than 5 per cent in the 1980s to 18 per cent in the early 1990s, and increased to 25 per cent by the end of the century. Across Europe, about 5 per cent of pneumococci isolates show resistance to penicillin, but the rates in specific countries can vary markedly: Spain, Romania and Israel have resistance rates of more than 25 per cent, for example, while in the UK, Germany and Sweden, among others, rates are 5 per cent or below. Resistance to the macrolide erythromycin has also been increasing in Europe, with a similar geographical pattern.

Acute earache (otitis media) is the most frequent illness for which antibiotics are prescribed for children in industrialised countries.

Vaccines can play a key role in preventing the emergence and spread of resistance in the community, by reducing the frequency of infection and consequent antimicrobial use. Efforts to develop effective pneumococcal vaccines began as early as 1911, fell away when penicillin seemed the answer in the 1940s, and then restarted in the late 1960s. A vaccine suitable for adults was developed in the 1970s, but recent efforts have focused on a newer vaccine, suitable for children, which was licensed in 2000. In addition to a dramatic reduction in the incidence of invasive pneumococcal disease, it was reported in March 2005 that the use of this vaccine in US children was associated with a reduction in macrolide resistance in S. pneumoniae, an encouraging result.

Professor Palmer wishes to clarify the relationship at the individual level by weighing up people’s past antibiotic use against their personal risk of developing a resistant infection. “The connection is not well documented,” he says. He has been tackling the problem by investigating the incidence of antibiotic resistance among patients with urinary tract infections (UTIs) at ten GP surgeries in south Wales. Initially, Professor Palmer and his team asked the GPs to request urine samples of all new cases of UTI, rather than selecting only the cases that were difficult to treat.

About a thousand new cases of E. coli UTI were identified and linked to each patient’s antibiotic history (i.e. which antibiotics were taken and when), and to the prescribing patterns of the general practices of the patients. Preliminary results showed that of the thousand cases investigated, half had infections with bacteria resistant to at least one antibiotic. “We want to try to find out what is the difference – what are the risk factors for antibiotic resistance,” explains Professor Palmer. “This is important because our data suggest that patients with a resistant infection have poorer outcomes and their normal activities are disrupted for longer.”
Half of those patients with a resistant UTI (around 25 per cent of the total) had had antibiotic treatments for a UTI in the past. Twenty per cent had had antibiotics for an upper respiratory tract infection, and 6 per cent for a skin infection. The strongest associations between previous prescription and resistance identified so far are for amoxicillin and trimethoprim, resistance being more likely if the doses were higher and the prescriptions more recent.

Do antibiotics given for respiratory infections (which are mostly viral) produce resistance in the bacteria that normally colonise that intestine, leading to a resistant UTI?

The next step is to find a causal pathway: why one person gets a resistant UTI while another does not. Do antibiotics given for respiratory infections (which are mostly viral) produce resistance in the bacteria that normally colonise that intestine, leading to a resistant UTI, for example? Or are people picking up resistance from someone else in their household?

The team also aims to work with GPs in evidence-based prescribing. “At present a lot of prescribing is done at the theoretical level: by the time the microbial test results come through, people with negative results have already received antibiotics,” says Professor Palmer. Worryingly, in a supplementary study of patients who sent in a urine sample, the team found that although 69 per cent of patients had been given antibiotics for their UTI, 45 per cent did not have bacteria in their urine. “This may be an untackled area of unnecessary prescribing,” says Professor Palmer.

Improving prescribing and use

Worries about rising levels of resistance to antibiotics in bacteria have focused on whether all prescriptions are necessary. Patients with an infection – particularly upper respiratory infections – often expect antibiotics, and can pressurise doctors to prescribe these even if they are unlikely to be effective. The pressures on both patient and doctor are quite understandable: an anxious parent, a sick child, and a doctor who may not be sure at the time whether the infection is caused by a virus or a bacterium. In the UK, antibiotic prescribing by GPs increased in the 1980s, peaked in 1995 and then fell by over a quarter between 1995 and 2000 (mostly owing to reduced antibiotic prescribing for acute respiratory illnesses).

One solution would be to help doctors when making their initial diagnosis. At present, bacteria samples from a patient have to be sent to a laboratory for testing – and the results may take several days to return. Quick, accurate tests that could identify patients with bacterial infections requiring an antibiotic would make a huge difference: if the test turned out to be negative, they would not be given unnecessary antibiotics and risk developing resistance. Some bacterial illnesses may not require antibiotics at all. For example, conjunctivitis affects one in eight school children every year, and in a recent study in Oxford found that chloramphenicol, the standard treatment, had little benefit: most children will get better by themselves.

Improving GPs’ awareness of a local link between rates of antibiotic resistance in their patients and their own prescribing decisions may also change prescribing patterns and reduce the emergence of resistance. But equally important are measures to educate patients in how to use antibiotics correctly. People often fail to finish the full course of treatment – as soon as they feel better, they stop and the leftovers sit in the medicine cabinet. The incorrect dosing can fail to eliminate the bacteria completely from the system and will encourage growth of the resistant strains.

Anti-tuberculosis drugs are two-edged swords. They destroy Mycobacterium tuberculosis but can also select for resistant bacteria, against which those drugs are then ineffective. The first anti-TB drugs – streptomycin, para-aminosalicylic acid and isoniazid – were introduced in the 1940s, and although resistance arose soon after, it was always to one drug alone.

By the end of the 1960s, rifampin had arrived, and its use in combination with other drugs led to a decline in drug-resistant and drug-susceptible TB in developed countries. Funding for and interest in TB control programmes also declined and, for the following 20 years, no systematic monitoring of drug resistance was carried out.

With the arrival of HIV/AIDS in the 1980s, the transmission of TB increased, including more outbreaks of multidrug-resistant TB.

The situation changed significantly with the arrival of HIV/AIDS in the 1980s. The transmission of TB increased, including more outbreaks of multidrug-resistant TB (MDR-TB) – strains resistant to isoniazid and rifampin. In response, the Global Project on Drug Resistance Surveillance was launched in 1994 to monitor trends in resistance. Its 1997 and 2000 reports showed that drug resistance was present worldwide and that the prevalence of MDR-TB ranged from 0 to 14 per cent of new TB cases (median: 1.4 per cent) and 0 to 54 per cent of previously treated cases (median: 13 per cent).

The recently published third report has data on 77 sites around the world, collected between 1999 and 2002. Significant increases in the prevalence of MDR-TB were seen in Estonia, Lithuania, Tomsk Oblast (in Russia) and Poland, but there were significant decreases in Hong Kong, Thailand and the USA. Most western and central European countries see only a few cases of MDR-TB each year but, alarmingly, it was estimated that two provinces in China (Henan and Hubei) see more than 1000 cases each year, and Kazakhstan and South Africa more than 3000 each.

Acquired and primary resistance

Unlike many other bacteria, M. tuberculosis strains cannot gain resistance by the transfer of mobile pieces of DNA containing ‘resistance genes’. Instead, resistance arises when spontaneous mutations in the genome cause changes in proteins that are either directly targeted by the drug, or that control its accumulation or activation in the cell. Such mutations are rare, and as the genes involved in resistance to various drugs are in different parts of the genome, the risk of a double spontaneous mutation is extremely low. Using combinations of drugs (as in the WHO-recommended directly observed therapy short-course programme) should therefore preclude the selection of resistant strains. MDR-TB develops when drugs are used individually or as part of inappropriate combinations. This results in selection of resistant bacteria within the individual (‘acquired resistance’) who can then spread the resistant strain to other people, who will develop MDR-TB as a result of ‘primary resistance’.

Debates have raged over the relative contribution of acquired and primary resistance to the burden of drug-resistant TB in different communities. The controversy focuses on whether MDR-TB strains can be transmitted between people or whether the mutations that confer drug resistance also impair bacterial survival or reproduction. A series of studies over the last decade, looking at MDR-TB in hospitals, among healthcare workers, in prisons and in communities, has provided evidence that MDR-TB can be transmitted and has shown that these dangerous bacteria are expensive to combat and constitute a major public health issue.
The most extensive MDR-TB outbreak reported to date occurred in New York, where there were 267 cases of infection by Beijing (or ‘strain W’) strains of M. tuberculosis. Since then, drug-resistant and susceptible Beijing strains have been found throughout the world; for example, a recent study has shown that they contribute, along with other strains, to the drug-resistant epidemic in the Western Cape area of South Africa. MDR-TB was first identified in the Western Cape area in 1985; within nine years it accounted for 25 per cent of the TB isolated in the region, and South Africa is now listed by the WHO as one of the high-burden countries for drug-resistant TB. Many other MDR-TB strains exist: smaller outbreaks involving such strains have arisen recently in the Czech Republic, Portugal and Norway.

**Breaking the cycle**

How can we stop the transmission of drug-resistant TB? Rapid diagnosis and new drugs would make a huge difference, and here our understanding of the genetics of M. tuberculosis is beginning to bear fruit.

At present, both of these possibilities are problematic. Once a sputum sample has been taken from someone suspected of having TB, it takes three to six weeks to grow the bacteria and confirm that they are indeed M. tuberculosis, and another two to three weeks to determine which drugs the strain is susceptible to. Such delays leave a critical window for MDR-TB transmission. The tests are also costly, and there are considerable problems with standardisation of tests and with stability of the drugs in different test media.

New, fast molecular tests are currently being evaluated. These have been designed to examine the bacteria’s DNA directly from sputum samples within seven days, looking for the specific mutations found in resistant strains. Future tests may well use DNA chips that look for multiple mutations at once, such as mutations in the rpoB gene, which can serve as a marker for MDR-TB, and mutations in the genes involved in resistance to isoniazid. Although these techniques have the potential to be automated and re-used, they are expensive only if they become cheap and robust are they likely to be used in resource-poor countries, where most drug-resistant cases occur.

Equally important is the development of new drugs for treatment of MDR-TB. Currently available ‘second-line’ drugs are more expensive and less effective than rifampin and isoniazid, and require more intensive clinical management. The hope is that the sequence of the M. tuberculosis genome, and advances in our understanding of the function of its genes, will provide a wealth of opportunities for target identification and drug discovery. This is of course a longer-term challenge. But MDR-TB is a manufactured problem and, with astute application of resources and expertise, a manufactured solution is within reach.

Terrie Victo (Medical Biochemistry, School of Health Sciences, University of Stellenbosch, South Africa) and Douglas Young (Centre for Molecular Microbiology and Infection, Department of Infectious Diseases and Microbiology, Imperial College London, UK) are supported by a Wellcome Trust Collaborative Research Initiative Grant to develop rapid tests for MDR-TB in South Africa.

Henry Nicholls reports on a project that is tackling drug-resistant tuberculosis in Uganda.

In the late 1990s, scientists working in Uganda began to notice that some patients receiving treatment for tuberculosis (TB) were not responding to conventional drugs.

A pilot study subsequently revealed an alarming statistic: in nearly a quarter of patients tested, the bacterium that causes TB had developed resistance to more than one of the drugs that would normally treat the disease.

With backing from the Wellcome Trust and the Burroughs Wellcome Fund, project leader Jerrold Ellner and his colleagues at the University of Medicine and Dentistry of New Jersey have teamed up with researchers at the London School of Hygiene and Tropical Medicine and scientists in Uganda. Their first mission has been to verify the extent of the problem. So far, they have recruited over 350 patients on the TB ward at Kampala’s main hospital, and aim to recruit 500 in all.

Initial results suggest that around 12 per cent of patients who previously had received treatment now have multidrug-resistant TB (MDR-TB). Although this is not as high as in the pilot study, it is still of concern. “These individuals are transmitting multidrug-resistant strains to other patients and health workers within the hospital and often take the disease back to their communities,” says Edward Jones, project coordinator.

Another aim of the project is to come up with new and faster ways to identify drug-resistant strains. “Currently, there is no routine testing for multidrug-resistant tuberculosis in Uganda,” says project member Ruth McNerney of the London School of Hygiene and Tropical Medicine. If these patients can be identified quickly, they could be isolated in an attempt to contain transmission and different drugs can be tried. Dr McNerney is exploring several ways to speed up the process of diagnosis. Molecular techniques, for example, could produce a result in a matter of days rather than months.

However, these advances in diagnosis have created a profound need. “We feel the ethical obligation to provide treatment to drug-resistant tuberculosis cases identified in the context of our research,” says Dr Ellner. If further work can come up with an affordable and effective treatment, the Ugandans should have a system in place to prevent the explosion of MDR-TB strains.

Dr Henry Nicholls is a freelance writer based in London, UK.
Antibiotic-resistant bacteria present huge challenges to healthcare in developing countries, as Jeremy Farrar and Richard Adegbola describe.

Walk into a local pharmacy in Vietnam, and you can buy antibiotics. Walk along a street in Lagos in Nigeria or Accra in Ghana, and hawkers will try to sell you cigarettes, sweets, penicillin, ampicillin and cephalosporins. In these and many other countries, antibiotics are uncontrolled and unregulated – easy to get and easy to misuse. With such wide availability, it is not surprising that the prevalence of bacteria resistant to one or more antibiotics has increased in many developing countries.

Even so, when access to drugs is easier than access to doctors, or when people may be able to pay for one but not both, the issue might not be quite so straightforward. As Dr Jeremy Farrar, Director of the Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam, points out: “When antibiotics are unregulated, you clearly get big problems with drug resistance. But we should not forget there may be benefits of easy access – people may be treated sooner. We need a greater understanding of anti-microbial resistance, and in working out how to deal with this problem we need to appreciate we live in the real world, where HIV, the incidence of the virus is still relatively low in Vietnam, around 1 per cent, which is much lower than in sub-Saharan Africa.

In Ho Chi Minh City, improvements in sanitation have led to marked reductions in typhoid, but in the Mekong delta, home to about 30 million people, the disease is still a major problem: incidence rates there are about 200 per 100 000 people. “Asia has the most resistant Salmonella typhi (the bacterium that causes typhoid fever) in the world, with major outbreaks in the Central Asian Republics, and ongoing endemic disease in both South and South-east Asia,” says Dr Farrar. “Typhoid is not as big a disease as tuberculosis or pneumonia, but treatment is very difficult – 95 per cent of isolates in Vietnam are resistant to all first- and second-line antibiotics. All cheap antibiotics are of no value at all; even the two drugs that do work, don’t work particularly well and they are very expensive.”

The Gambia
Unlike the situation in African countries such as Senegal and Nigeria, many bacteria in the small West African country of The Gambia show surprisingly low rates of resistance to antibiotics. Although the reasons are not clear, Dr Richard Adegbola at the UK Medical Research Council Laboratories in Banjul suggests that the low rates are due to control of and reduced access to drugs. “There is more control of how drugs get in and out of the country, and the management of drugs is very interesting compared to other countries,” he says. “Every child has a healthcare card that records every visit to the hospital and the antibiotics they have been prescribed. But access to antibiotics can be variable: as you move towards the east into the provinces, there are district hospitals that have few drugs. So there is less use, which may discourage the emergence of resistance.”

A study by Dr Adegbola and colleagues, aimed at checking the level of antimicrobial activity in urine before presentation at hospital, found that only 7 per cent of Gambian children under five had taken antibiotics. “This is very low,” he points out. “If you did this study in Bangladesh or China, say, it would be 80–90 per cent. Cheap drugs such as chloramphenicol still have their place in The Gambia.”

For S. pneumoniae, the bacterial pathogen that kills the most Gambian children, resistance to penicillin is almost unknown, whereas neighbouring countries show over 20 per cent resistance and many other countries have rates that top 50 per cent. Even so, resistance to chloramphenicol (about 8 per cent) is beginning to emerge, and in vitro resistance to cotrimoxazole (trimethoprim-sulfamethoxazole) is already over 80 per cent.

Events in neighbouring countries have also had an effect on antibiotic resistance in The Gambia. “Until about five years ago, tetracycline was still used in The Gambia to treat gonorrhoea, but it has been useless for well over 15 years in countries like Nigeria because the bacteria are highly resistant,” says Dr Adegbola. “Then there were crises in neighbouring countries, an influx of people into The Gambia, and we saw a sudden rise in resistance to penicillin and tetracycline. These drugs are now virtually useless in the treatment of this disease.”

Vaccines
With the power of antibiotics under threat, might new vaccines be the answer? The introduction into The Gambia of a vaccine against Haemophilus influenzae type B shows how effective they can be: the vaccine was introduced in 1997 and the bacterium, previously a major cause of pneumonia and meningitis, has now all but disappeared from the country. Between 2000 and 2004, Dr Adegbola and colleagues (led by Professor Felicity Cuts) trialled an S. pneumoniae vaccine that not only was effective in preventing pneumonia, but also reduced the number of deaths and hospital admissions in general. “Finding that the number of deaths and hospital admissions can be reduced the much can make a big difference with a government when you are trying to introduce a vaccine in Africa,” he points out.

In Vietnam, the Hospital for Tropical Diseases and the Oxford University Clinical Research Unit have been funded through a Wellcome Trust Technology Transfer grant that was awarded to Microcense Ltd to trial a typhoid vaccine. “Currently available typhoid vaccines give short-lived protection, are expensive, or require four doses orally,” says Dr Farrar. “We’re about to start phase 2 and phase 3 trials of this new one-dose oral vaccine.”

Dr Jeremy Farrar is Director of the Wellcome Trust-funded Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. Dr Richard Adegbola is Head of Bacterial Diseases Research Programme at the UK MRC Laboratories, Banjul, The Gambia.
There are two ways to launch a biological assault on the human race. The first is to evolve a lethal bacterium or virus against which we have no defence. AIDS, Ebola and Marburg’s disease are examples caused by toxic organisms that have cut a swath through humanity and spread fear and panic.

Less feared but just as deadly are organisms that have found a way round our defences by evolving protection against the antibiotic drugs we use to destroy them. In evading destruction they survive to multiply, infect and ultimately overwhelm us. It is their indestructibility, rather than their toxicity, that makes them lethal.

The collective blindness the world has shown to the growth of these drug-resistant bacteria is a matter of immense concern. We have seen the consequences in the UK with outbreaks of methicillin-resistant Staphylococcus aureus (MRSA) that have closed hospital wards, caused extensive suffering to patients and a rising death toll. Deaths from MRSA almost doubled from 487 in 1999 to 955 in 2003.

Those in the know have long warned that we are heading for a public health disaster. So the high profile given to MRSA by politicians of all parties should have been welcome. The Conservatives led the charge with their demand for cleaner hospitals, and Labour responded with new pledges and new targets.

Cynics will question why the problem of hospital infections, which is decades old, should have seized politicians and the public now. Opportunism is the simple answer. In an election year, with little separating the parties on health policy, the chant of ‘cleaner hospitals’ proved an easy slogan with which the Conservatives could attack the Government.

It suited the media, too. “NHS killer bug shock”, the Sun screamed in a typical front-page headline on 22 March 2005, above an account of an investigation into MRSA levels in one hospital. It was a scurrilous story of the kind newspaper editors love; it was simple to understand and it offered another stick – other than long waiting lists (which are coming down) – with which to beat the NHS.

But there was justification, too. The UK’s record on MRSA is undeniably poor, with one of the highest infection rates in Europe. At the time of writing, the most recent figures show that there were 3519 bloodstream infections with MRSA in the six months from April to September 2004.

That represented a fall of 6.3 per cent on the same period a year before, and the Government claimed it was a “turning point” when the figures were published in March. But the Conservatives accused ministers of “election trickery” for publishing six-monthly figures covering last summer rather than the normal 12-monthly total. The overall figure is expected to rise in the next six-month period from October 2004 to April 2005 because it covers the winter.

The problem is worst in the south of the country and worst in specialist hospitals that treat sicker patients than general hospitals. The Department of Health published figures for every NHS trust, showing some had cut their MRSA rates while others had seen big rises.

To tackle the menace, ministers promised a crackdown on poor hygiene with hospitals ranked in a league table of cleanliness. But it is too little, too late.

Two years ago, Sir Liam Donaldson, the Chief Medical Officer, ordered every NHS trust to appoint a director of infection control with responsibility for cutting deaths and illness caused by superbugs. Last year, the National Audit Office (NAO) launched an investigation and found rates of infection still rising.

It criticised the NHS for failing to implement many of the recommendations it had made four years earlier and called for a more robust approach to antibiotic prescribing and hospital hygiene.

One thing distinguished the NAO report from most pronouncements by politicians. It acknowledged how intractable the problem of tackling hospital infections is, estimating just 15 per cent might be preventable.

The best defences against the bacteria are frequent handwashing, aseptic techniques and minimising use of intravenous lines and catheters, which provide access for bacteria. Hiring more cleaners and getting them to mop more thoroughly, desirable as that is for other reasons, has little effect. Yet this is the solution favoured by politicians and the press.

There are grounds for hope. The Dutch successfully reduced their MRSA rate by implementing a policy of “search and destroy”, which involved screening patients for the infection and isolating those found to be positive in single rooms in modern hospitals. To do the same here will be harder because MRSA rates are higher and the pressure on beds greater. We should have acted as the Dutch did, in the early 1990s, when infection rates were still low.

For one thing, however, we can be grateful. The political spotlight that has been shone on MRSA has given new impetus to the drive to tackle it. Success is long overdue. Superbugs may harm far fewer people than, say, heart disease, but patients have a right to expect they will get better – not sicker – when they go into hospital.

Jeremy Laurance is Health Editor at the Independent.
WHERE ARE THE NEW DRUGS?
The pharmaceutical industry and antibiotic development

Why is it that when resistance to antibacterial agents is increasing, fewer agents are being developed? By Jeff R Edwards.

The golden age when the pharmaceutical industry made frequent significant new entries to the anti-infectives market has ceased. New drugs to tackle human pathogens have become much harder to find: many existing drug classes are exhausted or near to exhaustion, and genomic-based technologies have yet to deliver new agents. This discourages commercial infection research, as do ever-escalating regulatory requirements.

Financial considerations
The pharmaceutical industry has been criticised for withdrawing from or significantly reducing its infection research. There is truth in this, but critics often miss two key points: big pharma has provided every antibacterial agent on which the world’s physicians rely; and not all companies have pulled back. Among those still active, AstraZeneca and Johnson & Johnson both have significant antibacterial research groups and also devote effort to tuberculosis research.

Pharmaceutical companies are always under considerable commercial pressure, and it is important to note that anti-infectives are not highly profitable. Few have annual sales greater than US$500 million, while many therapies for chronic disorders have sales four to ten times greater. This factor becomes very apparent within a company when there is competition for core resources, particularly those from new chemical series, can provide that balance.

Taking advantage of the pressures on big pharma, many small companies requiring more modest financial returns have emerged. Typically, they have excellent scientists restricted by lack of finance and, on occasion, by lack of development experience. Even so, they may be the future home of anti-infective research and development. This is exemplified by Cubist’s in-licensing and developing daptomycin, a drug previously shelved by a large company.

Regulatory issues
Regulatory agencies require that sponsors adopt the highest standards in all studies. Exemplary standards should not be questioned, but the magnitude of studies continues to increase. Fortunately, some progress has been made on the international harmonisation of requirements. Once toxicological hurdles are passed, human tolerance and kinetic studies can commence. If these progress to therapy trials, the demands are stringent, exceptionally costly and, for some clinical indications, almost impossible to meet. This exacting position for the assessment of known drug classes already acts as a disincentive to commercial research and development; one can but speculate about the caution with which new chemical types will be viewed.

Bugs and drugs
Statements that there are no new drugs and that resistance is out of control are misleading. More accurately, there are few new significant agents being developed and, while in vitro surveys show an increase of less susceptible bacteria, only a small proportion of patients is infected with untreatable bacteria. Thus, the current situation is that physicians have less choice of agents: all of the established therapies retain significant clinical utility, but we should expect this situation to worsen.

Producing variants of existing drug classes used to be an effective development strategy, but has now become far more difficult. Drug classes such as beta-lactams (e.g. penicillins) are, or soon will be, exhausted – as is the case with quinolones (e.g. ciprofloxacin). Other established classes such as glycopeptides (e.g. vancomycin) and macrolides (e.g. erythromycin) are also still yielding new analogues, the clinical value of which remains to be established.

Newer agents are emerging. Linezolid, the first of a truly new class, the oxazolidinones, and daptomycin, a lipopeptide, both have novel mechanisms of action and address our current problems with methicillin-resistant Staphylococcus aureus (MRSA). Other agents are being developed that none exhibits comprehensive activity against difficult Gram-negative bacteria such as Pseudomonas, Acinetobacter and Burkholderia spp., which now are untreatable in a proportion of infections. Finding compounds active against these bacteria has been a significant challenge for decades.

When success comes, as it surely will, we will have novel compounds acting on new antimicrobial targets

With our increasing knowledge of the genomes of human pathogens, target-based research is hailed as the most likely technology to reveal new chemical classes. This cutting-edge technology identifies genes that are essential to a bacterium and is the starting point for identifying or designing compounds that can interfere specifically with the protein products of these genes. However, multiple difficulties have to be overcome to allow progress from ‘essential gene’ to development status, and the technology has been oversold. Consequently, many research groups, including major companies, have prematurely withdrawn their activities, leaving us ‘target-rich but compound-poor’. However, when success comes, as it surely will, we will have novel compounds acting on new antimicrobial targets. One must hope that this novelty will be marketable.

Concluding comments
With time, bacteria have developed resistance to all antibacterial agents. A flow of new agents, designed to circumvent these problems, has up until now provided the prescriber with alternatives. It is unfortunate that common bacteria such as staphylococci (MRSA) are becoming still more problematic and that Pseudomonas and Acinetobacter spp., for which we have never had many effective agents, are now more common and cause untreatable infections. Also, it is regrettable that this is occurring when pharmaceutical resources are being directed to other therapy areas. This impacts not only on the availability of new agents but also on sponsorship of academic research in this important area. It is imperative that a formula for making anti-infectives attractive to the pharmaceutical industry is re-established; without its expertise new agents are much less likely to emerge.

Dr Jeff R Edwards spent his career within the pharmaceutical industry and now runs a consultancy, JEIC, in north Wales.
“The problem of antibiotic resistance isn’t going to go away,” says Dr Tim Walsh at the University of Bristol. “The problem of the ‘MRSA superbug’ is well documented but there are other bacteria, such as Pseudomonas aeruginosa and Acinetobacter spp., which are multi-resistant and cause serious infections. Unfortunately, while the problem warrants increasingly urgent attention, it is attracting less. “The number of large pharmaceutical companies working on anti-infectives has dropped around 60 per cent from ten years ago,” says Dr Walsh. As patients only take antibiotics for one or two weeks for serious infections – unlike other therapies such as anti-depressants, which are taken on an ongoing basis – it makes less economic sense for pharmaceutical companies to invest in developing them.

This is not to say that antibiotic development has stopped altogether. For example, three new anti-infectives with good activity against MRSA – daptomycin, linezolid and tigecycline (FDA approved June 2005) – have been or will be available worldwide. “Plus there are other drugs in phase 2 and 3 [clinical] trials, so new treatments for MRSA and bacteria causing community acquired pneumonia are well advanced in the pipeline,” says Dr Walsh.

Breaking the Gram-negative barrier

Unlike Gram-positive bacteria, Gram-negatives have an additional membrane that water-soluble (hydrophilic) compounds cannot unconditionally traverse. Drugs must cross the membrane through pores – channels that enable nutrients and metabolites to enter the cell. However, many Gram-negative bacteria, especially Pseudomonas, Acinetobacter and Enterobacteriaceae, can shut off some of these pores and prevent foreign compounds from entering.

As a result, points out Dr Walsh, although there are many compounds oriented towards Gram-positive bacteria such as MRSA on the market, is there anything available that is purely anti-Gram-negative or, at least, has good activity against pan-resistant Acinetobacter and Pseudomonas spp.

An answer might lie in an agent produced in our bodies by Gram-positive bacteria such as MRSA on the market, which is not harmful. Dr Walsh became interested in this possibility after work by Dr Malcolm Gallagher at the University of Liverpool on cystic fibrosis patients. He found that where antibiotics inhibited normal Gram-positive flora, there was a growth of the Gram-negative pathogen Pseudomonas aeruginosa. These virulent pathogens cause inflammation in the lung in cystic fibrosis, killing many young adults with the disease. “So, in our own bodies, a Gram-positive bacterium produces a factor that inhibits the growth of Gram-negative bacteria such as P. aeruginosa which are resistant to just about all antibiotics,” says Dr Walsh. “Such observations go back to the days of Fleming who found that penicillin on a plate (produced by Penicillium spp.) inhibits Staphylococcus aureus.”

The Bristol team found that the factor was produced by the Gram-positive bacteria Streptococcus mitis, Streptococcus oralis and Streptococcus mutans, bacteria that live in the human respiratory tract and mouth. They then grew a protein ‘soup’ produced by these bacteria and challenged Pseudomonas bacteria (obtained from samples from people with cystic fibrosis) to find out what factors – produced by the soup – kill the intransigent P. aeruginosa.

Investigations revealed that S. mitis produces at least two proteins that inhibit P. aeruginosa: the peptides RTA1 and RTA2. These have a number of specific properties that could make them ideal anti-Gram-negative compounds. First, they are very small and thus more likely to bypass the immune system (larger peptides are more likely to elicit an immune response). Second, both are hydrophilic and soluble at very high concentrations. And third, importantly, both actively break open and kill P. aeruginosa. “A lot of drugs need to be killed in the presence of disinfectants or penicillin and many antibacterial agents are destroyed by the membrane of P. aeruginosa,” says Dr Walsh. “But this protein breaks open and kills P. aeruginosa.”
prevent the organism from growing, but don’t actively kill it,” explains Dr Walsh. “RTA1 and RTA2 punch holes in P. aeruginosa bacteria, in their membranes.”

Having isolated RTA1 and RTA2 and determined the genome region responsible for their production, the team now aims to modify and maximise the ideal therapeutic properties of these peptides. “We want to make them shorter, increase their hydrophilic properties and make them more active,” says Dr Walsh. “The smaller one, RTA1, is fantastically active against Gram-negative bacteria; it’s a molecule well worth pursuing.”

So far, the team has tested the peptide on only ten pan-resistant clinical isolates, but aim to examine its potential against Gram-negative bacteria. “As with any new drug, we want to see whether it works, and make sure it isn’t too toxic to the patient,” says Dr Walsh.

On the positive side

Although compounds targeting Gram-positive bacteria are more readily available than those directed at Gram-negative bacteria, many Gram-positive bacteria, such as MRSA, are becoming resistant to antibiotics, especially in hospitals.

“There’s a big unmet clinical need for new agents,” says Dr Michael Dawson at Novacta Biosystems Ltd in Hatfield, Hertfordshire. Dr Dawson originally helped to set up the start-up company in 2003, in Hatfield, Hertfordshire. Dr Dawson originally helped to set up the start-up company in Norfolk in 2003, to take advantage of technologies for engineering biosynthetic pathways using molecular biology, in order to optimise the therapeutic potential of natural products against infection.

“We’re interested in a molecule that hasn’t been used before and has no cross-resistance with other antibiotics – including vancomycin, which people use against MRSA when other antibiotics have failed,” he says. The molecule in question, called mersacidin – meaning, literally, ‘kill MRSA’ – has good activity against MRSA as well as other Gram-positive pathogens.

Like many antibiotics, mersacidin is made by bacteria (in this case, a bacillus) to kill other bacteria competing with them in the environment. But Dr Dawson points out that it has a different mode of action from other antibiotics. “It kills the microorganism by binding to lipid 2, a monomer that builds up the cell wall,” he explains. Lipid 2 is also a target of vancomycin, but mersacidin binds to a different part of the molecule.

Mersacidin was discovered in the early 1990s by the pharmaceutical company Hoechst, who considered developing it for its antibacterial activity. At that time, however, resistance was less of a problem and, despite its promising properties, the molecule needed ‘improving’ in some areas to give it a spectrum of activity. The complex chemistry required for this task was not then available.

Researchers at the University of Bonn have since cloned the pathways in the bacillus bacteria that make mersacidin, and Novacta Biosystems has developed and patented methods of manipulating the biosynthetic apparatus. “We’re using molecular biology technologies to modify the bacillus organism and get it to make lots of variant molecules that can be tested for their effectiveness against MRSA,” explains Dr Dawson.

The team aims to test thousands of these variants, looking at which pathways they kill and at which concentrations. Although still at the lead improvement phase at the moment, in two years’ time they hope to start conducting phase 1 trials of mersacidin with human volunteers.

Dr Tim Walsh at the University of Bristol, UK, and Dr Michael Dawson at Novacta Biosystems Ltd in Hatfield, UK, are funded by awards from the Wellcome Trust’s Technology Transfer Division.

Where to find out more

A selection of internet resources about antibiotic resistance and healthcare-associated infections. Most sites include links to additional resources.

- **General**
  - Bugs and Drugs on the Web
    - www.antibioticresistance.org.uk
  - Centre for Disease Control and Prevention
    - www.cdc.gov/ndss/antimicrobial_resistance/facts.htm
  - UK Department of Health: Healthcare-associated infection
    - www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/HealthcareAcquiredInfection/fs/en
  - Department of Health: Antimicrobial resistance
    - www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/AntimicrobialResistance/fs/en
  - Health Protection Agency: Antimicrobial resistance
    - www.hpa.org.uk/infections/topics_az/antimicrobial_resistance/menu.htm
  - British Society for Antimicrobial Chemotherapy
    - www.bsac.org.uk
  - Hospital Infection Society
    - www.his.org.uk
  - Other
    - European Antimicrobial Resistance Surveillance System
      - www.ears-rvm.nl
    - US Food and Drug Administration: Antibiotic resistance
  - For children
    - The Bug Investigators
      - www.buginvestigators.co.uk
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