The food chain: Antibiotics use in food animals

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Wellcome Focus
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 use 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 Wellcome News Supplements – such as last year’s, Taking 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).

Dr Giles Newton, Science Editor
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 useful, 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 play 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 human-kind’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 gramicide 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 isolated 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 use, produced actinomycete-derived drugs for companies. A core that he called tetracycline. The price of penicillin, however, had been contested to the present day.

Medical uses on human patients were harder to limit – even though, from the 1940s, fears that public enthusiasm would promote the selection of resistant strains did lead to some constraints. In Britain, the Penicillin Act of 1948 was explicitly intended to, for the first time, limit through prescription the public’s access to a drug that was not a poison. Nonetheless, during the 1950s, a penicillin-resistant strain of Staphylococcus aureus termed 80/81 infected hospitals and maternity wards across the world. Next to penicillin, drugs were introduced, post-operative infections proved common, and blame was cast on weaknesses of hospital cleaning in a culture of increasing dependence on antibiotics and changing nursing practices. The life of the actress Elizabeth Taylor was rescued after she was treated for staph pneumonia during the shooting of the film Cleopatra.

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 of Science at the University of Cambridge. His book on the history of penicillin is currently in press.

The medicine chest

Wellcome

2005

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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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 tuberculous, 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, churned out by bacteria, antibiotics tend to be harmless to people and animals. Some antimicrobials inhibit cell growth, disabling the drug. As a result of this strategy, the semi-synthetic agent metronidazole 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 vancomycin-resistant bacteria have been reported (see below).

Researchers sifted through 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 defences 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, disrupt 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 peptidoglycan 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. Pencillin 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.

Although the fungi-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 carbapenicillin (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.

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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 to the drug.

The 1970s were the halcyon days of antibiotics: bacteria were vanquished and infection rates were slashed. No one seemed to mind that how the drugs worked remained a mystery. Today, as antibiotic-resistant bacteria have become a worldwide problem, Dr Tenson agrees that figuring out how antibiotics work is a priority. “This is the time to re-investigate several issues that were left open during the 1970s,” he says.

His research has examined how antibiotics target ribosomes, the protein-making factories in cells. “The ribosome is especially fascinating — it is the target for many important antibiotics,” says Dr Tenson. If antibiotics bind to ribosomes and hinder their action, protein manufacture stops and the bacterium dies.

Dr Tenson focused initially on erythromycin and its relatives, a widely used class of antibiotics known as macrolides. Normally, in a bacterium, the growing protein emerges from the ribosome through a tunnel-like structure. Macrolides interrupt this process by binding to the tunnel opening. “The peptides cannot enter the tunnel and the ribosome is inactivated,” he explains.

But how does this blockage eventually kill the bacterium? When the macrolide binds to the ribosome, it stalls peptide production and the nascent peptide remains attached to transfer RNA (tRNA), the transporter molecules of amino acids, without leaving. Since tRNA is usually recycled in the cell, this leads to a tRNA shortage, which is probably what stops the bacterium growing. “This is new and unexpected, because it is the missing link,” says Dr Tenson.

Dr Tanel Tenson is at the Institute of Technology, Tartu University, Estonia, and is a Welcomes Trust Central European Senior Research Fellow.
Rather than confine obsolete antibiotics to the scrap heap, scientists are finding ways to rejuvenate them.

At the University of Durham, a team of biochemists led by Adrian Walmsley is focusing on the pumps that bacteria use to eject antibiotics, siphoning drugs out of the cell so that they never reach toxic levels. “Pumps are one of the main mechanisms of resistance. If we could develop pump-blockers, we could use those in a cocktail with the original drugs,” says Professor Walmsley.

One advantage to targeting pumps is that the blocking agents could approach the cell from the outside. “A major constraint in drug development is that to attack cytosolic proteins you have to get the drug into the cell,” he says. “You need it to be hydrophobic enough to get into the cell, but also soluble enough to get it into tablet form.” As this combination is hard to achieve, many antibiotics need to be given intravenously. Drugs that target membrane pumps would overcome this constraint.

Another way round the resistance problem would be to stop bacterial pump production by interfering with genetic controls. “If you can design a repressor that blocks those transcription factors on DNA, you’d be able to switch off expression of the pumps,” Professor Walmsley points out. To his surprise and delight, the same compounds that block pumps also bind these transcriptional regulators – a potential double whammy. If this approach proves successful, it could resuscitate a host of valuable antimicrobials that have ceased to cure infections, but the more they are used, the greater the chance of resistance arising and spreading.

Bacteria employ several devices to stave off antibiotic attack. Some antibiotics must accumulate inside the bacteria to be effective, and bacteria have pumps on the cell membrane to expel the drug before it has a chance to reach its intracellular targets. If a mutation allows a bacterial cell to overproduce membrane pumps, it will eject the drug faster than it can diffuse in. The drug concentration inside the cell will remain low, and the bacterium will survive.

A chill ran through the clinical world when, in 1997, scientists discovered that an MRSA strain had picked up vancomycin-resistance genes. Even vancomycin is losing its power. Bacteria have developed or acquired alternative ways to make their walls, a complicated task that involves a cluster of mutant genes working together. 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 peptidoglycan chains are free to line 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 strain 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 may be a drain on energy that the cell may, 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. If the 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 baumanni 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.
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. 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 ‘MRSA’ was highlighted in big red letters) – not just in the pleural fluid but also in the nose. They hadn’t in the nose as well. They hadn’t 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 fluid but also in the nose. They hadn’t 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.

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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.”

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, we’ve all probably got it, 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.

When I went on a ventilator for a week. There were several attempts to take me off but I became agitated, which affected my breathing, so I had to go back on. I was delirious during my time in HDU – 33 days in all – and remember very little of it. It was like a dream.

At this stage I became very ill. An infection in my chest spread to my lungs and I needed oxygen, a chest drain and a tracheotomy to help me breathe. I was also put on a ventilator for a week. There were several attempts to take me off but I became agitated, which affected my breathing, so I had to go back on. I was delirious during my time in HDU – 33 days in all – and remember very little of it. It was like a dream.

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 Klebsiella pneumoniae. 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.

KNOW YOUR ENEMY

Tracking resistant strains of bacteria

Older methods, such the comparison of patterns of DNA fragments in a technique called pulsed-field gel electrophoresis, are very good for comparing strains within a single laboratory but are not well suited to comparisons between laboratories. In contrast, MLST results can be posted on internet databases that hold a huge amount of information, readily examined or added to by researchers worldwide. For any one species, the database can hold all the sequences ever identified for each gene fragment; the known sequence types and their allelic profiles; the properties of individual isolates of each sequence type; and basic information such as country of origin, type of disease and whether the bacteria were found in a hospital or in the community.

Using multilocus sequence typing, the spread of particularly virulent or resistant strains can be tracked, their origins traced and the routes of disease transmission understood.

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.

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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 *S. 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 homosexuals 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 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.

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**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 where 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 multi-resistant 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 these are 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 live harmlessly 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, injectable 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 systematic 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 Hepworth and his team in the Antibiotic Resistance Monitoring and Reference Laboratory report on the resistance of pathogens to the most commonly used antibiotics.

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“MRSA IS NOT A SUPERBUG”
A doctor’s view of MRSA

Graeme Wilson, a consultant respiratory physician, argues that MRSA is weak compared with other disease-causing agents, and not our greatest worry.

“Alexander Fleming warned us that the more we expose people to penicillin, the greater the risk of antibiotic resistance. It is less of a problem in the UK than elsewhere in the world, because we can only get antibiotics here on prescription: people cannot just walk into a pharmacy and buy them. And in UK hospitals, we have microbiologists to guide us in prescribing the most appropriate antibiotics for particular cases.

But patients are still being given unnecessary antibiotics. They may come into hospital with a chest infection exacerbating an underlying condition, such as chronic obstructive pulmonary disease, and get put on pneumonia antibiotics – which they do not need because they have not got pneumonia. Doctors have a real responsibility to tailor antibiotic use as narrowly as possible to a particular patient situation. If we kill only the organism that is causing the disease, resistance is less likely to develop.

Unfortunately, this is easier said than done. We cannot always culture and target the initial infecting organism, so we have to give broad-spectrum antibiotics. As an example: in 30 per cent of cases of community-acquired pneumonia – which they do not need because they have not got pneumonia. Doctors have a real responsibility to tailor antibiotic use as narrowly as possible to a particular patient situation. If we kill only the organism that is causing the disease, resistance is less likely to develop.

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Instead, we are still being given unnecessary antibiotics. They may come into hospital with a chest infection exacerbating an underlying condition, such as chronic obstructive pulmonary disease, and get put on pneumonia antibiotics – which they do not need because they have not got pneumonia. Doctors have a real responsibility to tailor antibiotic use as narrowly as possible to a particular patient situation. If we kill only the organism that is causing the disease, resistance is less likely to develop.

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Life on the ward
Antibiotic resistance in hospitals

Every week, hospitals treat thousands of people – of all ages and with diverse illnesses and injuries. A huge quantity of antibiotics is prescribed to treat or prevent bacterial infections, and it is in hospitals that the intense relationship between antibiotic use and the development of antibiotic resistance in bacteria is most evident. Development of resistance is only part of the problem, however. Resistant strains of bacteria have spread in and between hospitals, partly under the pressure of antibiotic use but sometimes because infection control has been less effective than it could have been.

Unfortunately, for a variety of reasons, methicillin-resistant Staphylococcus aureus (MRSA) is now endemic in many UK hospitals and other healthcare settings such as care homes. Large ‘specialist’ hospitals face particular problems as their patients tend to be sicker and more prone to being colonised or infected by typical hospital pathogens. The spread of resistant bacteria such as MRSA is preventable, as countries such as Sweden, Denmark and The Netherlands have shown, with their successful use of a tactic known as ‘search and destroy’. In the UK, however, infrastructure and bed occupancy rates have hampered efforts: many UK hospitals do not have isolation facilities, and bed occupancy has risen to such high levels (more than 90 per cent of beds are occupied by patients at any one time) that it is extremely difficult to separate patients from one another and so prevent the spread of antibiotic-resistant bacteria. Thus, ‘search and destroy’ control measures have been undermined by the way in which patients are typically managed in the UK.

While almost all infections with antibiotic-resistant bacteria can still be treated, there is evidence that outcomes are worse in some cases. For example, death rates in people with bloodstream infections of MRSA are twice those seen in people infected with susceptible S. aureus strains.

Infection control
Isolating patients and scrupulous hand hygiene – such as with alcohol-based hand rubs – appear to help slow the spread of bacteria. Indeed, the plateauing of UK MRSA rates in the last three or so years may be owing to improvements in these areas (although the bacteria themselves may have reached a threshold). But much of infection control practice is based on empiricism – experience rather than data holds sway. Effective ways of preventing cross-infection and the spread of antibiotic-resistant pathogens still need to be defined and/or refined. Crucially, these approaches may need to differ depending on whether a particular antibiotic-resistant pathogen has already become established (i.e. is endemic) or whether it is rare. A good analogy here is plugging the holes in a leaking dyke: eventually more than fingers will be needed to sustain the barrier.
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.

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 sepsis – 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 \textit{Staphylococcus aureus}

Why have two strains of methicillin-resistant \textit{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 \textit{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 bloodstreamz, and an older, sicker, more vulnerable population is being treated. Someone comes along having treated a patient with MRSA, their fingers touch the catheter of a different patient, MRSA comes along having treated a patient with MRSA, their fingers touch the catheter of a different patient, MRSA comes along.”

The two strains, epidemic MRSA-15 and -16, seem to be particularly well suited to life in hospitals. They can live for up to six months on surfaces, are easily transmitted between people, and cause infections around wounds as well as life-threatening conditions such as bloodstream infections, pneumonia and infection of the heart lining. Today, in UK hospitals, more than 40 per cent of \textit{S. aureus} infections are resistant to methicillin, other penicillin-class antibiotics and often additional antibiotics.

Doctors have still had the glycopeptide vancomycin as backup but, worryingly, resistance to this ‘drug of last resort’ has emerged in the last few years. “Intermediate resistance was identified in Japan in 1967, and these isolates have now been found in every country,” says Dr Enright. “And there have been three separate isolates of full vancomycin resistance found in the USA in 2002 and 2004. So resistance to the newest antibiotics already exists.”

Tracking MRSA

Using multilocus sequence typing (MLST; see page 13) to ‘DNA profile’ different strains of \textit{S. aureus}, Dr Enright is seeing where they are descended from and how and why they fit into families. “Data from more than 1000 isolates of \textit{S. aureus} from 15 different countries are 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.”

Not all cases of MRSA arise in hospitals. Community-acquired MRSA seems to cause disease in children and young adults, and is rare in the UK (only about 100 cases have been identified in the last three years) but more prevalent in the USA, mainland Europe and Australia. The strains involved are resistant to fewer antibiotics than hospital MRSA, but produce a dangerous toxin (called PVL) that can lead to skin infections such as large boils or clusters of boils (up to 10 cm in diameter in some cases) and deep-seated abscesses. If the bacteria get into the lungs, which is fortunately a rare event, a devastating pneumonia that kills more than 40 per cent of patients can result.

Tracking the opportunist

Methicillin-resistant \textit{Staphylococcus aureus}

Within the genome sequence of \textit{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 Mark Holden.

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

The genomes of two \textit{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 \textit{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 MSSA476 are found in a community-acquired strain called MIV2 (whose genome has also been sequenced), closely related to MRSA252 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.

Not all cases of MRSA arise in hospitals. Community-acquired MRSA seems to cause disease in children and young adults, and is rare in the UK (only about 100 cases have been identified in the last three years) but more prevalent in the USA, mainland Europe and Australia. The strains involved are resistant to fewer antibiotics than hospital MRSA, but produce a dangerous toxin (called PVL) that can lead to skin infections such as large boils or clusters of boils (up to 10 cm in diameter in some cases) and deep-seated abscesses. If the bacteria get into the lungs, which is fortunately a rare event, a devastating pneumonia that kills more than 40 per cent of patients can result.

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, but this is not the case.” Instead, some community isolates turn out to be descended from a penicillin-resistant strain of \textit{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.

Dr Matt Holden is at the Wellcome Trust Sanger Institute, UK.
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-infecting bacteria — is leading governments to cut back animal antibiotic use.

All of the different types of antibiotic that are used in animals are also used in humans

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.

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.

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.

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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.
BACTERIA AT LARGE

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, bloodstream infection and meningitis, as well as sinuses 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; however, in the 1960s, the first intermediate penicillin-resistant pneumococcus of clinical relevance was isolated in Australia, and in 1974, the first report of infection due to a fully penicillin-resistant pneumococcus was described in the USA.

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 pneumococcus 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.

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Urinary tract infections

High degrees of antibiotic usage have often been linked to increased levels of resistance. This is the case both internationally (in countries where antibiotics can be bought over the counter, resistance is thought to be higher than elsewhere) and locally (GP practices with high prescribing rates are said to experience higher resistance). But such associations do not measure individual exposure to antibiotics: are the patients receiving antibiotics the same ones from whom antibiotic-resistant bacteria are isolated? ‘A lot of assumptions are made about antibiotic resistance in the scientific literature,’ says Professor Stephen Palmer at the University of Wales. ‘And often they aren’t based on good evidence.’

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.’

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.

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TACKLING THE WHITE PLAGUE
Multidrug-resistant tuberculosis

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.

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.

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 (Hunan 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.

Tommy Victor and Douglas Young discuss how molecular approaches are increasing our knowledge of drug-resistant tuberculosis (TB).
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 of South Africa. MDR-TB was first identified in the Western Cape area in 1985; within nine years it accounted for 3.5 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.

**Rapid diagnosis and new drugs would make a huge difference**

**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 Victa (Medical Biochemistry, School of Health Sciences, University of Stellenbosch, South Africa) and Douglas Young (Centre for Molecular Microbiology and Infectious Disease, 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 and use the drugs we have.”

Dr Farrar believes there are several factors that contribute to resistance in Vietnam. For example, 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 but one antibiotic.”

Vietnam resistance to antibiotics is at worrying levels for three bacteria that Dr Farrar picks out as having the most impact on Vietnam’s healthcare: Streptococcus pneumoniae, Mycobacterium tuberculosis and Salmonella typhi. The first of these, often termed the pneumococcus, causes the most common kind of bacterial pneumonia and meningitis are responsible for about 1.6 million deaths each year.

“Of community-acquired pneumonias that lead to death, the pneumococcus is probably the number 1 bacterium, and resistance is a highly significant problem,” says Dr Farrar. “We found that 60–70 per cent of bacteria carried by people living in urban parts of Vietnam are resistant to the penicillins; resistance to third-generation cephalosporins is lower but increasing and these patterns are now emerging in bacteria that cause invasive disease such as pneumonia and meningitis. The situation is getting worse, and Vietnam is certainly one of the countries with a major problem.”

Tuberculosis is a massive problem in Vietnam. The country has one of the highest incidence rates in the world, despite a well-implemented treatment programme. “In the urban centres, the rates of TB are as high as anywhere in the world,” says Dr Farrar. “There are also very high rates of resistance to one drug and, worryingly, to multiple drugs.” Although TB rates are higher in partially unregulated use is the norm and unlikely to change. New imaginative strategies to prevent the further spread of resistance are required as there are few if any new and affordable antibiotics on the way. In some diseases there is certainly the possibility of complete resistance developing and a return to the pre-antibiotic era.

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 very 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) trialed 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 Microceutics Ltd to trial the 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.
MRSA, politics and the press

Jeremy Laurance examines why.

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 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.

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 development resources, which typically will support a chronic-therapy opportunity. Yet a successful business should seek to have a balanced portfolio and have some contingency for a ‘megabrand’ failure; infection therapies retain significant clinical utility, but we should expect this situation to worsen.

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; 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 but 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, JEC, 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, there is nothing 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 from normal flora and 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...
whether it works, and make sure it isn’t too toxic efficacy (and any subsequent derivatives) on disparate pan-resistant clinical isolates, but aim to examine its So far, the team has tested the peptide on only ten negative bacteria; it’s a molecule well worth pursuing.” and make them more active,” says Dr Walsh. “The genome region responsible for their production, the Having isolated RTA1 and RTA2 and determined the therapeutic properties of these peptides. “We want to take advantage of technologies for engineering to set up the start-up company in Norfolk in 2003, in Hatfield, Hertfordshire. Dr Dawson originally helped to modify the bacillus organism and get it to make 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 pathogens 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 mersenacidin with human volunteers.

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

UK Department of Health: Healthcare-associated infection
www.dh.gov.uk/PolicyAndGuidance/HealthAndSocial CareTopics/HealthcareAcquiredInfection/fs/en

Department of Health: Antimicrobial resistance
www.dh.gov.uk/PolicyAndGuidance/HealthAndSocial CareTopics/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.hs.org.uk

Other

European Antimicrobial Resistance Surveillance System
www.ears-rvm.nl

US Food and Drug Administration; Antibiotic resistance
www.fda.gov/ccp/opacom/hottopics/anti_resist.html

For children

The Bug Investigators
www.buginvestigators.co.uk

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