by Wendy Pyper

the rise and rise of resistan

Only 50 years since antibiotics were hailed as miracle drugs against potentially fatal bacterial infections, a new breed of 'superbugs', resistant to many antibiotics in our arsenal, is on the rise.

Multiple drug resistant (MDR) strains of *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Pneumococcus*, *Enterococcus*, *Salmonella*, *Klebsiella* and *Pseudomonas*, are appearing in hospitals and communities worldwide, thanks to years of antibiotic use and abuse in human medicine, animal husbandry and agriculture.

Concern over this trend was expressed by a panel of speakers at the International Congress of Bacteriology and Applied Microbiology in Sydney last August.

One speaker, Professor Julian Davies from the Department of Microbiology and Immunology at the University of British Columbia, said more than one million tonnes of antibiotics had been released into the environment in the past 50 years, exerting a selective pressure on microbes to adapt or die.

But after nearly four billion years on earth, microbes are experts at adaptation. Antibiotic resistant genes, for example, originally evolved to counteract natural antibiotic production, a key defence in many a microbe's survival strategy. Fifty years of human intervention, and the development of synthetic antibiotics, is a small hurdle in microbial evolution – a 'minor crisis' from which many bacteria are emerging victorious.

The rise of these so-called 'superbugs' has been aided by our inability to internationally regulate antibiotic use in animal husbandry and human medicine.

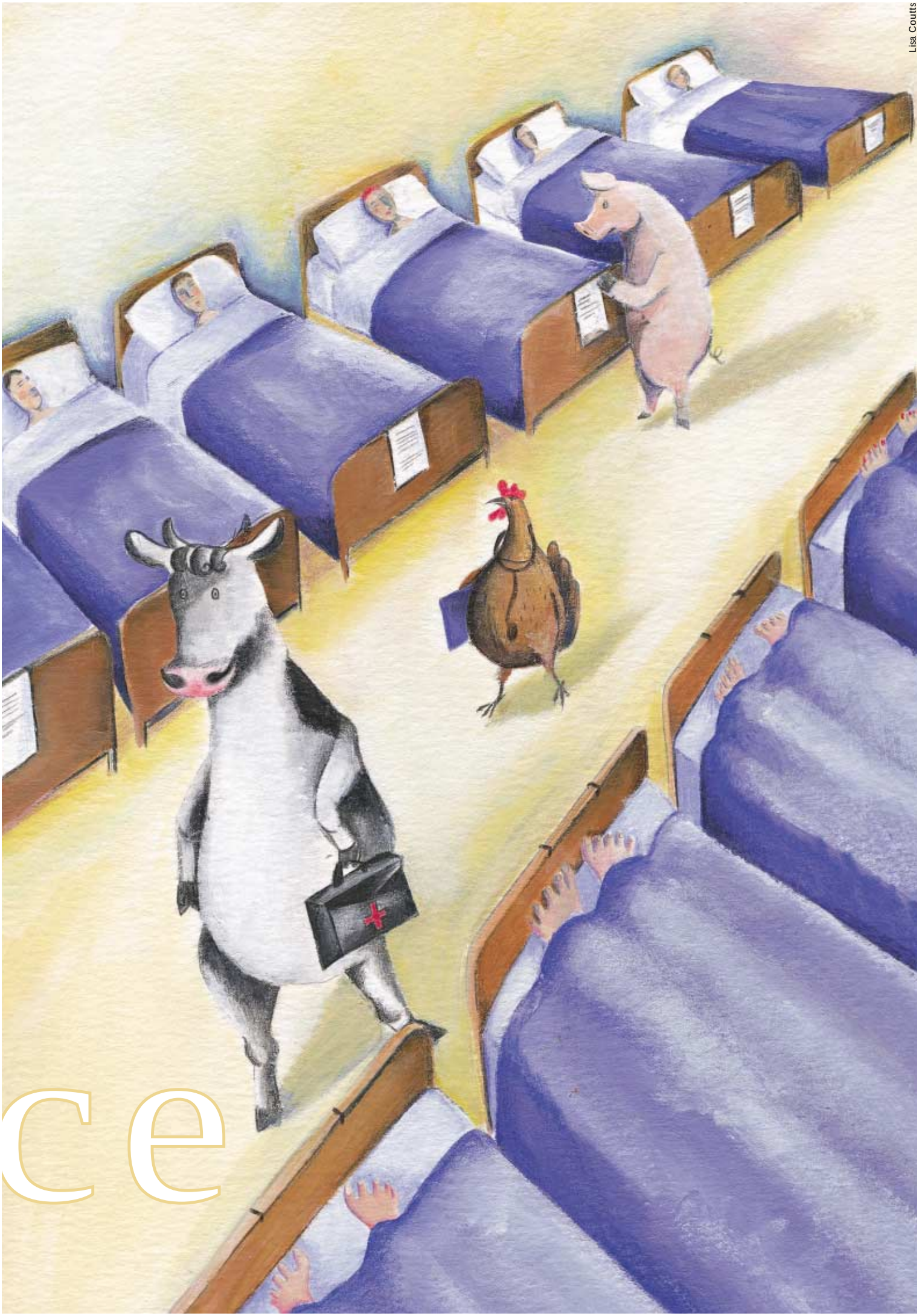
Of particular concern are antibiotics used for 'growth promotion' in the intensive production of cattle, poultry and pigs. These are given in low or 'subtherapeutic' doses to improve feeding efficiency so that marketable weights are reached sooner, and with less food.

Growth promoters are thought to work by controlling low levels of pathogenic bacteria, which normally would divert the animal's energy from weight gain to fighting infections. But they also create a selective pressure for resistant bacteria in the animal gut and it is feared these bacteria may pass their resistance genes on to human bacteria, via the food chain.

As far back as 1968, the Swann Committee to the British Parliament found compelling evidence that resistance genes could move from animal bacteria to human bacteria. The committee recommended that antibiotics used in human medicine should not be given to animals as growth promoters.

Later European studies also found evidence of resistance gene transfer, as well as cross-resistance of animal bugs

10.1071_ISSN0311-4546EC102p14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Lisa Coutts

Vaccines: the last line of defence

WITH ANTIBIOTIC resistance on the rise, threats of bioterrorism, and the emergence of one or two new infectious diseases every year, the world may seem like a dangerous, inhospitable place.

But director of the CRC for Vaccine Technology, Professor Michael Good, says predictions of doom and gloom must be tempered by the promise of developing technology.

'It's important to consider new advances in technology which can prevent or treat infection, and one of the most exciting areas is that of vaccine development,' he says.

Vaccines have played a key role in the control of infectious diseases in the past, wiping out smallpox and protecting against potentially fatal diseases such as measles, tetanus and whooping cough. In the future, vaccines could become our only weapon against diseases such as malaria, HIV, TB and antibiotic-resistant infections, which have triumphed over other forms of prevention and treatment.

'Vaccines are looking like the last line of defence against some of these organisms,' Good says. 'There's no vaccine that's been developed so far that organisms have been able to evade.'

Vaccine development utilises a number of strategies depending on the type of organism targetted. For example, whole dead or 'attenuated' organisms are used for the smallpox vaccine, a modified toxin or 'toxoid' is used for organisms such as tetanus, and 'subunit' vaccines – pieces of an organism – are used for influenza and hepatitis B.

While these strategies are tried and true, they do not work for all organisms. Research into new strategies such as synthetic peptides, recombinant proteins and naked DNA is necessary to open up new opportunities for disease control.

'As we learn more about vaccine technology, we can develop new technology and give ourselves more opportunities,' Good says.

Unfortunately, money for vaccine research, and health research in general is hard to come by, especially when developing countries are the target market. Good says Industry has withdrawn from vaccine research because the commercial return is poor. Given that it takes some 15 years and $300 million to make a vaccine, the commercial risk is high. But the health benefits and economic return a successful vaccine generates, is even higher.

'As well as saving lives, vaccines result in significant cost savings,' Good says. 'In the US alone, vaccine development saves $10 billion a year. So they are the most profitable way to improve human health and save lives.'

However, even with great vaccines, infectious disease control is difficult if people don't get vaccinated. According to a 1995 Australian Bureau of Statistics report, only 53% of children aged between three months and six years were fully immunised against a range of potentially fatal diseases. While there is no single reason for this decline, apathy, confusion or forgetfulness is they most likely cause. Some may object to the risks associated with vaccination, but Good says the risks are very small.

'Typically you can get pain at the site of injection, low-grade temperatures and irritability,' he says. 'But there are very few major side effects, and they are so rare that when they do come up, it's very difficult to demonstrate that they're associated with the vaccine.'

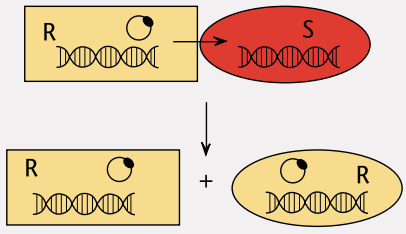
Whatever the reasons, continued public education in the importance of immunisation is required, to avoid new epidemics of vaccine-preventable diseases. Basic research into the lifecycles of disease-causing organisms, and improvements in public housing and infrastructure around the world, will also go a long way to ensuring a healthy future.

to antibiotics important in human therapy. But Australia, the United States and most countries in the European Union (EU) continued to use human antibiotics as growth promoters, arguing that any risk to human health was largely theoretical and exceedingly small.

In 1997, the EU banned the use of the controversial glycopeptide growth promoter avoparcin and in 1999 suspended the use of four others, based on the 'precautionary principle'. Given the money and livelihoods invested in the production and sale of antibiotics, this move had many critics who believed the evidence for resistance transfer was insufficient to justify such a ban.

The debate has now moved to Australia. In 1998 the Australian Federal Government set up the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR), made up of health, veterinary, molecular biology and primary industry representatives, to review antibiotic use in the Australian livestock industry (see story on page 19)

Here too, opinion is divided over the risk growth promoters pose to human health. Medical and scientific representatives say that our potential to treat life-threatening human infections is diminishing as a result of resistance transfer from animal bacteria to human



Bacteria can transfer genes to other bacteria. In this case, an antibiotic resistant bacterium (R) transfers the gene(s) for resistance to an antibiotic sensitive (or susceptible) bacterium (S) which becomes resistant as a consequence.

If more than one resistance gene is on the piece of DNA that is transferred, the sensitive bacterium becomes resistant to several different antibiotics in one step.

bacteria. Veterinary and industry representatives say the evidence for this transfer is weak and that the real culprit is years of antibiotic misuse in humans.

So what is the evidence so far?

Mobile genes

Research in the 1960s showed that bacteria share their genetic material through a process called 'horizontal gene transfer'. This involves the direct exchange of genes between bacteria, or via bacterial viruses or free floating, 'naked' DNA.

Dr Ruth Hall from CSIRO Molecular Science in Sydney, and a member of JETACAR, says the movement of genes between bacteria is facilitated by mobile genetic elements called 'plasmids'. These tiny loops of DNA are independent of the bacterial chromosome, and are freely exchanged by 'conjugation', where a 'donor' bacterium sends a copy of the plasmid down a thin tube into the 'recipient' bacterium.

Hall's research, which looks at how resistance genes attach to plasmids, has shown they tend to congregate at sites on the plasmid called 'integrons', maximising their ability to spread.

'Antibiotic resistance genes line up in rows in integrons,' she says. 'So a plasmid with one resistance gene can gain a second and a third, and so on, and when that plasmid moves between bacteria, all the resistance genes go with it.'

This means that one plasmid could carry resistance genes to both human and animal antibiotics, effectively destroying the idea that antibiotics not used in humans could be safely used in animals.

'Choosing to use antibiotics in animal husbandry, which are not used in human medicine, sounds logical,' Hall says. 'But it doesn't work. If you select for resistance to an antibiotic not used in human medicine, the same resistance gene or one sitting right next to it, may be a resistance gene for something that is used in humans.

'We've known for a long time that resistance genes tend to congregate together on plasmids. The scientific community wonders how it can be that this simple but important fact has been ignored for so long.'

Hall's revelation is alarming, particularly as more than half the antibiotics used in Australia go into animal husbandry, mainly for growth promotion and prophylaxis.

Not worth the risk

Professor Peter Collignon, from the Department of Infectious Diseases and Microbiology at Canberra Hospital, says many people don't realise that resistance to one antibiotic will have implications for every other antibiotic in the same group or class.

For example, the glycopeptide growth promoter avoparcin is a close relative of the 'last line of defence' human antibiotic, vancomycin.

'Avoparcin is really vancomycin by another name,' says Collignon, who is also a member of JETACAR. 'In Australia, vancomycin was used to treat resistant *Staphylococcus* in humans, 15 years before avoparcin was approved for growth promotion in animals.'

'Gene transfer from animal bacteria to human bacteria only has to happen once, anywhere in the world, to enable the bacteria containing the newly acquired gene to spread throughout the human population.'

Studies in Europe have now linked avoparcin to the selection and amplification of vancomycin-resistant *Enterococci* (VRE), the cause of potentially life-threatening infections in humans. A similar situation exists with the streptogramine class of antibiotics, which have been trialled as a treatment for VRE. Because one type of streptogramine, virginiamycin, has been used as a growth promoter for 30 years, new related human drugs are encountering resistance.

Unfortunately, the importance of these particular drugs in human medicine was not realised until the emergence of resistant human pathogens, some years after their registration for animal use. Collignon says the use of such last-line antibiotics in animal husbandry should not have continued.

'Last-line human antibiotics shouldn't be used in animals at all, and particularly not as growth promoters,' he says. 'This won't compromise animal welfare, because there are lots of other antibiotics that we can use to treat sick animals. But using valuable resources such as avoparcin limits our options for human drugs.'

What's more, Collignon says the available data suggests there is little or no benefit in growth promoters. Yet Australia 'squanders' non-renewable resources such as avoparcin in the quest for economic profit.

The quantities of avoparcin used in animal husbandry are at least 15 times greater than the quantities of vancomycin issued for human use. And at what cost?

'The savings may be worth 2c/kg in pigs, but in my view that's not worth

the cost of having 'superbugs' like VRE spread through the food chain and circulating in the general population,' Collignon says.

Dr Peter Holdsworth is the director of scientific and regulatory affairs in animal health at the industry lobby group, Avcare (the National Association for Crop Production and Animal Health). He says growth promoters have been assessed by the National Registration Authority in relation to their impact on human health, and that their use complies with regulations underpinned by legislation.

In terms of the quantities of antibiotics used in animal husbandry, Holdsworth says it's important to remember there are 19 million people in Australia compared with 500 million food producing animals, 'so it's not unrealistic to expect there to be more antibiotics going into animals than humans'.

He says growth promoters provide environmental benefits by reducing the amount of food and water required by the animals and by reducing manure and urine production through better digestion.

'And you can't ignore the fact that there have been six independent reviews overseas in the last four years that have looked at the same data and come up with different conclusions,' Holdsworth says.

'Some have identified a risk to human health, others have concluded that growth promoters pose no immanent risk to health. Until the risk is quantified, we don't really know if the recommendations put forward by JETACAR are proportional to the risk.'

Similarly, Dr Kevin Doyle from the Australian Veterinary Association (AVA) and a member of JETACAR, says the evidence that resistance genes can move from animal bacteria to human bacteria isn't strong.

'While these things can occur, the extent is an important question and this is quite difficult to measure,' Doyle says.

'For example, bacterial contamination of meat is destroyed by cooking unless some uncooked product is cross-contaminated during the cooking process.

'Enterococci in particular inhabit the human intestinal tract very temporarily and gene transfer has to take place in this short period. This is possible, but it is a limited opportunity when compared with antibiotic use in hospitals, especially when the vulnerability of patients is taken into account.

'However, we accept that we should be precautionary, and agree there should be an intensive scientific review to see whether the level of contribution by animal use to resistance in humans justifies a total ban.'

'If we ban the use of antibiotics for growth promotion and prophylaxis, we'll remove up to 50% of the antibiotics used. Surely, that's a good start.'

Bringing home the **bacon**

AS ANTIBIOTICS have played a major role in animal husbandry for such a long time, there are concerns that farmers will be unable to raise animals under modern conditions without them.

But Dr Ruth Hall says the banning of growth promoters in Europe did not lead to a collapse of the industry, and UK chicken producers have voluntarily ceased using five antibiotics. 'Detailed studies in one of their own facilities showed no advantage in antibiotic use,' Hall says.

Dr Kevin Doyle suggests that stricter controls and greater responsibility and discipline in antibiotic prescription would be a better solution than banning them. Most antibiotic growth promoters are now available in pre-prepared food, but JETACAR has recommended they come under veterinary prescription, where they will be subject to the ethics and accountability of professionals.

Professor Peter Collignon says improved animal housing and diet, and a change in the way we deal with disease, would go a long way towards reducing the need for antibiotics. This has been demonstrated in piglets in Europe where improved housing conditions saw a 30% drop in antibiotic use.

'Antibiotics ought to be a last resort,' Collignon says. They ought to be an indication that our methods aren't as good as they should be. We've got to improve our techniques and look at alternatives such as probiotics and new vaccines to stop infections occurring in the first place.'

Doyle says Australia has been at the forefront of research into vaccines for animal diseases for some time, but there are some diseases, such as necrotic enteritis in broiler chickens and enteritis in weaner pigs, for which vaccines are unavailable.

Hall says that 'while Australia has an excellent record in vaccine development, the use of antibiotics has gone up not down'.

But is this desire to put a figure on the frequency of resistance gene transfer just a way of avoiding the issue? Hall says that by the time the risk is quantified, levels of resistance will be too high and the consequences irreversible.

'What risk is quantitatively enough for them to think there is a need to act?' she asks. 'Every respected report says there is a risk. The frequency of gene transfer is irrelevant. What matters is not how often an event happens, but if it can happen.'

Gene transfer from animal bacteria to human bacteria only has to happen once, anywhere in the world, to enable the bacteria containing the newly acquired gene to spread throughout the human population. This is exemplified by the spread of antibiotic resistant *Staphyloccoccus, Salmonella* and *Enterococcus* around the world through international travel and trade.

Hall says maintaining the status quo will not only risk human health, but also the health of the environment. Waterways now contain detectable levels of antibiotics through run-off from farms and human effluent. And resistant bacteria are spread across paddocks and crops when manure from feedlots is used as fertiliser.

'We've got the world bathed in a dilute solution of antibiotics and it's changing our bacterial ecology,' Hall says. 'We simply can't afford to kill off microbes that are doing good jobs making the soil healthy and cleaning up rivers.

'If we ban the use of antibiotics for growth promotion and prophylaxis, we'll remove up to 50% of the antibiotics used. Surely, that's a good start.'

Shotgun approach

While the debate over antibiotic use in animals continues, both sides agree that the inappropriate use of antibiotics in human medicine is a major factor in the development of antibiotic resistance.

A 1997 study by the Royal Australian College of General Practitioners found antibiotics were still commonly prescribed for respiratory infections, many of which were viral and non-responsive to antibiotics. This is a worldwide problem according to Collignon.

'Estimates by the Centre for Disease Control say that between 50–60% of antibiotics don't need to be prescribed,' he says. 'But it is difficult to know whether something is a viral or bacterial infection. So there's an acute need to get better diagnostic tests that can help at the time of seeing a patient.'

The problem is exacerbated by the use of many top-line and broad-spectrum antibiotics which are prescribed for conditions where penicillin or a more targeted antibiotic would do just as well.

This is particularly apparent in hospitals where multiple drug resistant strains of *Staphylococcus aureus, Streptococcus pneumoniae* and *Enterococcus faecium* (VRE) have already claimed lives. Between 1994 and 1998, 71 cases of VRE causing infections were recorded in Australia.

Patients contribute to the problem when they don't complete their prescriptions, allowing bacteria to linger and strengthen their resistance. These bacteria can then be transmitted to others who may never have contacted the drug, through poor food handling, hospitalisation or poor hygiene.

'Antibiotics are the one drug that have a side effect potentially on the person taking them, but also on everybody else in society,' Collignon says. 'By amplifying or letting resistance develop, you can spread resistance to people even if they've never had the drug.'

Collignon says Australia needs to follow a set of guidelines and rules for antibiotic use that involve Government, and international standards to ensure local decisions are not undermined by travel and trade.

'The Health Department and the Agricultural Department can't do this by themselves. We need rules and policies that protect these precious resources for as long as possible. We need provisions in all areas to enable access to antibiotics when appropriate, and we need rules to reserve certain antibiotics for human use,' he says.

'But if we do all the right things in Australia, then import food containing antibiotic resistant organisms, we'll put a big hole in our wall. So we have to realise this is an international problem that needs an international approach.'

Report calls for **tighter** control

THE JETACAR report, *The use of antibiotics in food-producing animals: Antibiotic-resistant bacteria in animals and humans*, details 22 recommendations in five categories. These are:

- regulatory controls to ensure responsible use of antibiotics in food-producing animals;
- monitoring and surveillance of the use of antibiotics and changes in antibiotic resistance patterns;
- infection prevention strategies and hygiene measures to reduce the need for antibiotics;
- education, including prudent-use codes of practice; and
- further research into antibiotic use and alternatives to antibiotics.

A steering committee comprising members of the departments of health and agriculture has been established to examine the JETACAR recommendations. The report is available on the web at http://www.health.gov.au/pubs/jetacar.htm. For a printed copy of the report, contact: (02) 6289 5887.

Abstract: The rise of antibiotic-resistant bacteria has been aided by our inability to internationally regulate antibiotic use in animal husbandry and human medicine. Of particular concern are antibiotics used for 'growth promotion' in the intensive production of cattle, poultry and pigs. European studies have found that resistance genes can move from animal bacteria to human bacteria and various growth promoters have been banned from use. In Australia, medical and scientific representatives say our potential to treat life-threatening human infections is diminishing as a result of resistance transfer from animal bacteria to human bacteria. Veterinary and industry representatives say the evidence for this transfer is weak and that the real culprit is years of antibiotic misuse in humans. A committee set up to review antibiotic use in Australia has recommended tighter controls, monitoring and education.

Keywords: antibiotics, microbes, antibiotic resistance, growth promoters, resistance genes, bacteria, gene transfer, JETACAR, animal health, avoparcin, vancomycin, *Enterococcus, Staphylococcus, Streptococcus*.