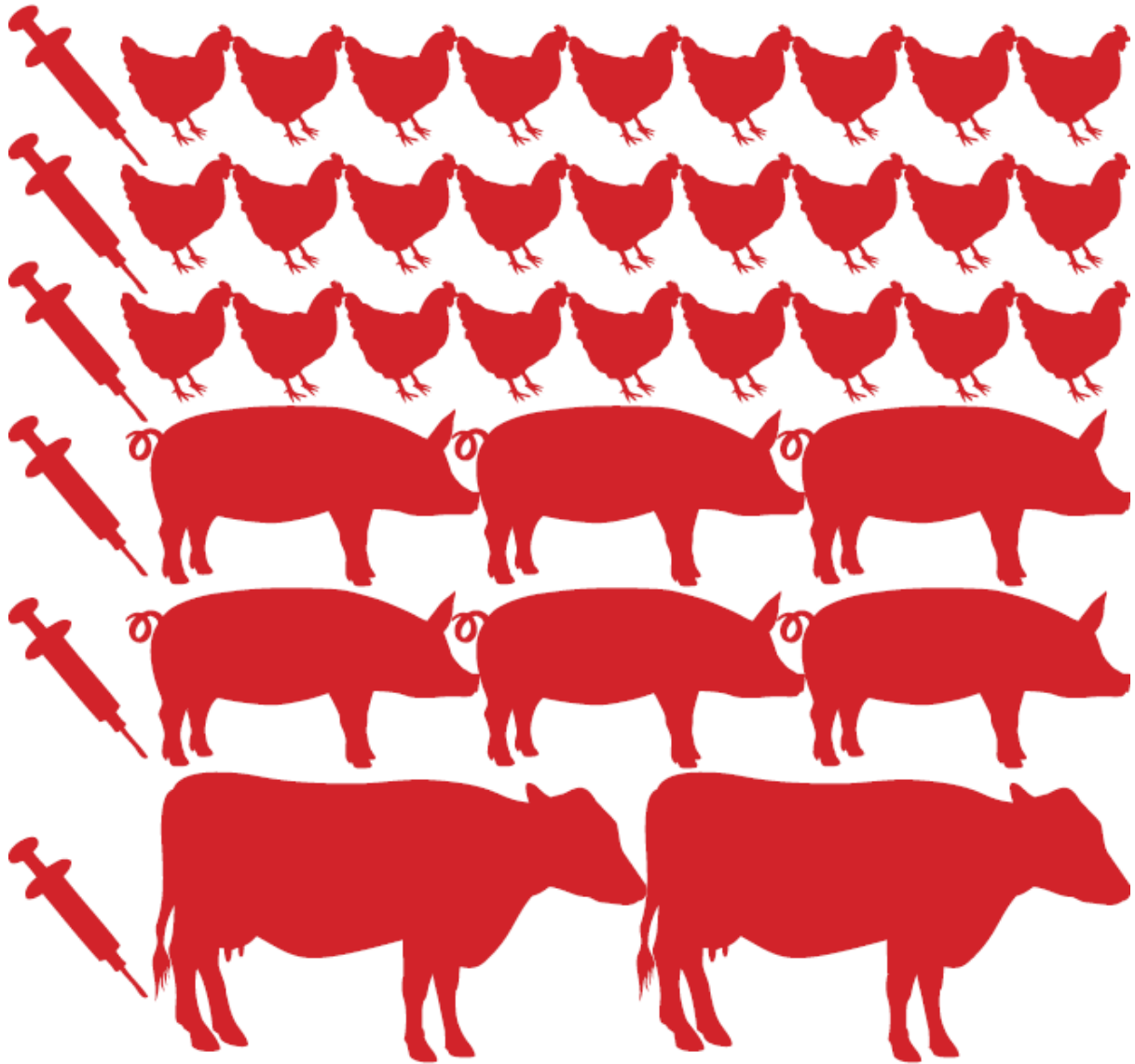


E.coli superbugs

on farms and food



E. coli superbugs – on farms and food

Cóilín Nunan and Richard Young

Report 6 in the series

The use and misuse of antibiotics in UK agriculture

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Preface

Antibiotics have revolutionised modern medicine and saved millions of lives; there are times when we have all been grateful for them to restore our own health, or that of loved ones. But problems can emerge with their over-use.

One of the major concerns to emerge in connection with such over-use is new *E. coli* and MRSA superbugs on farms. Most public health experts are agreed that resistant bacteria are created in food animals by antibiotic use and that some of these are being transmitted to people.

Antibiotic use on farms only contributes to a limited range of resistance problems in humans. However, evidence is building that for some infections the development of antibiotic resistance on farms is a significant part of the problem which makes it more difficult for doctors to treat affected patients, with potentially fatal delays in identifying an effective antibiotic when needed.

Organic standards, as certified by the Soil Association and others, prevent the routine use of antibiotics for animals. By providing the highest welfare and free-range conditions for our animals we can show that antibiotic use is required far less often. Several British studies, detailed in this report, confirm that this leads to very much lower levels of antibiotic-resistant bacteria on organically reared animals.

High antibiotic use in non-organic systems is often needed because the crowded conditions of intensive farming make the spread of disease amongst animals hard to prevent; as a result nearly 50% of all antibiotics consumed in the UK are used in farming. This report includes a detailed Soil Association analysis of the Government's own statistics, which alarmingly indicates that the overall use of antibiotics per animal on farms in the UK is now 18% higher than it was a decade ago.

That is why the Soil Association and our colleagues in the Alliance to Save Our Antibiotics (Compassion in World Farming and Sustain) are campaigning to raise awareness about this issue with the farming and public health communities. While we welcome voluntary initiatives, such as that by the British Poultry Council, to stop using cephalosporin antibiotics, we believe the time is right to strengthen the regulatory framework around the routine use of antibiotics on our farms, and to stop advertising antibiotics to farmers.

This report reviews a large volume of scientific evidence to provide an up to date and comprehensive analysis of what is known about the development and spread of a new highly antibiotic-resistant form of *E. coli* from farm animals to humans. We hope it will be widely read and that its findings and recommendations will be acted upon. We welcome both constructive comment and criticisms, all of which will be carefully considered.

The Soil Association's Strategy, *The Road to 2020*, outlines our plans to improve best practice in farming, and to work across the organic and non-organic sectors to achieve this. This report opens with endorsements from the academic and scientific community which acknowledge that antibiotic use represents a major issue that needs to be tackled. We hope we can work to build a coalition across all sectors to see if we can find solutions on this together, improving animal and human health at the same time.

Helen Browning,

Chief Executive

Summary recommendations

1. The UK's regulatory system for farm antibiotics was designed to limit the level of antibiotic residues in food and needs significant upgrading to address the issue of antimicrobial resistance as well.
2. The current over-reliance on the use of antibiotics on intensive farms cannot be resolved by farmers singlehandedly. Public health experts, the NHS, retailers and consumers should be actively involved in considering how to improve the situation, along with the Government, veterinary surgeons and farmers.
3. It should be recognized that a move towards higher welfare and less intensive production systems has the potential to reduce the use of antibiotics in agriculture significantly.
4. The preventative use of antibiotics in healthy animals should be phased out, and the overall use of antibiotics on farms should be halved within five years.
5. The use of modern cephalosporins and fluoroquinolones should be greatly reduced and their off-label use prohibited.
6. The UK should immediately prohibit the advertising of antibiotics to farmers. Advertisements to veterinary surgeons should be purely factual and not emotive in any way.
7. Action is needed to prevent calves drinking milk containing residues of modern cephalosporin antibiotics, as this can encourage the rapid spread of ESBL *E. coli* on farms.
8. Testing should be undertaken to establish the levels of ESBL bacteria on food. Funds should be made available to maintain and enhance the surveillance of ESBL *E. coli* on farms.

Foreword

Worldwide, rates of antibiotic resistance are increasing rapidly in bacteria that commonly cause disease. This results in increased deaths and suffering in people who develop serious infections with these bacteria. This problem is escalating much more quickly in Gram-negative bacteria such as *E. coli*. On some occasions there may be no antibiotics that work at all. There are no new antibiotic classes in the research and development pipeline for Gram-negative bacteria, so we need to preserve the antibiotics we have now for as long as possible.

A lot of resistance problems we see in people are the result of overuse of antibiotics in people and less than optimal infection-control practices which allow resistant bacteria to spread more easily from person to person. In the developing world, poor water supply also facilitates the spread of resistant bacteria from people to people, and between sectors - such as from animals to people and vice versa.

While much of the problem to do with antibiotic resistance in people is due to factors in the health system, there is also a significant contribution from the other sectors where antibiotics are used in very large quantities, and in particular from the agricultural sector. It is well established that in developed countries bacteria that cause predominantly gastrointestinal infections such as *Campylobacter* and *Salmonella* almost exclusively come across to people directly and indirectly from food animals, and this is also the case when these bacteria are antibiotic resistant.

E. coli is the commonest bacterial pathogen affecting people. It not only causes very common infections such as those in the urinary tract, but more importantly blood-stream infections (often colloquially known as blood poisoning). Serious infections with *E. coli* occur in hundreds of thousands of people in Europe every year, including in the UK. Worldwide there is mounting evidence that show that antibiotic resistance to critically important antibiotics in people, such as 3rd generation cephalosporins and fluoroquinolones, are related to the use of these drugs in food animals, with antibiotic use in poultry disproportionately increasing the risk to people. *E. coli* are acquired by all of us every day from foods. A study from the US suggested that more than 50% of all the antibiotic-resistant *E. coli* carried by people are derived from food animals. In the Netherlands, a large proportion of the antibiotic-resistant *E. coli* causing serious blood-stream infection are derived from food-animal sources. These resistant bacteria are associated with an increase in mortality and morbidity (such as prolonged hospital stay) compared to infections in people with antibiotic-sensitive strains. Increasingly the global evidence shows that a proportion of the human infections (and thus resistance) in *Staphylococcus aureus* (e.g. MRSA) is also related to the use of antibiotics in food animals, particularly last line antibiotics such as 3rd generation cephalosporins and fluoroquinolones.

What is not clear is the exact proportion of infections in people that arise from food animals. It would appear however that we have been significantly underestimating the contribution in many common bacterial pathogens, particularly *E. coli*.

Thus it is very important that we stop multi-resistant bacteria developing in food animals to prevent their spread to people. To do that we need to address the issue of inappropriate use of antibiotics in agriculture, just as much as in the health profession.

I welcome this review of the available scientific evidence from the Soil Association. It shows there is a clear risk to human health associated with the use of the critically important antibiotics in food animals,

and that resistant *E. coli* are transferred to people and cause serious infections that are more difficult to treat as a result.

What we now need is action by our Politicians, Governments and Regulators to do more to protect people from what is a clearly established hazard and an increasing risk. We also need large corporations such as supermarkets and Fast food chains, like McDonalds, to help combat this increasing problem. Unfortunately the wheels of Government and policy change can be very slow, and often the quicker and better protection of consumers can result from large buyers of these products insisting in contracts that certain agents are no longer used.

Peter Collignon AM

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Professor, Canberra Clinical School. Australian National University.

Statement from Dr Ron Daniels – the UK Sepsis Trust

Antimicrobial resistance is a major human health issue. In the context of a lack of investment in the development of new classes of antibiotics, and with the witnessed increasing incidences of both severe infections and antibiotic resistance over the last two decades, antimicrobial resistance will become increasingly problematic.

Severe infections in humans, or those which are immediately life-threatening, are characterised by the spread of bacteria or their toxins into the blood stream. Blood poisoning, more correctly known as sepsis, claims approximately 37,000 lives every year in the U.K. In the U.S.A, sepsis now accounts for more hospital admissions than heart attacks. Patients with severe sepsis have a one in three risk of death.

The most effective strategy in reducing deaths from sepsis is the reliable delivery to patients of appropriate antibiotics within the first hour following presentation. Healthcare organisations have responded to this by creating guidelines for the appropriate prescription of antibiotics when patients present with infection.

These guidelines must balance the need to provide adequate treatment with the need to combat the rise in antibiotic resistance. Stewardship requires that guidelines be designed to treat the more common bacteria causing such infections rather than over-treating with the most broad-spectrum agents, with the result that resistant organisms are frequently not covered by initial therapies. This risk of inadequate cover leads patients with sepsis to be twice as likely to die as if adequate cover were given. Conversely, as the frequency of resistant bacteria rises, guidelines change to provide a broader spectrum of cover, in some cases recommending the use of the antibiotic group carbapenems to which certain species are already developing resistance. The fear of inadequate cover, coupled with the tendency to use broader and broader spectrums of cover, will inevitably lead to worsening antimicrobial resistance patterns.

Antibiotic resistance is developing faster than we can develop new antibiotics- if we don't act now, we will rapidly arrive at a situation where we are unable to treat some bacterial infections.

The body of literature supporting the theory that resistant bacteria arising in animal populations can transfer to and colonise humans, and that genetic material from bacteria present in farm animals can transfer to bacteria normally present in humans, is overwhelming. It is now certain that agricultural, veterinary and food industry use of antibiotics – which represents one half of all antibiotic use in the UK – impacts on antibiotic resistance in animals which in turn impacts on antibiotic resistance in humans. It follows that the resultant increase in the antibiotic resistance patterns of bacteria in humans has a direct impact on mortality in humans.

The relative lack of regulation around the selection of type of antibiotic for administration in farming and veterinary medicine, coupled with the risk of injudicious use of antibiotics to bolster profit, is costing human lives. If we do not act soon, it will have contributed to a situation where we have no effective antibiotic to offer. I wholeheartedly support this effort by the Soil Association to nurture more judicious and regulated antibiotic use.

Dr Ron Daniels

Consultant in Critical Care Medicine, Sutton Coldfield

Executive Director: Global Sepsis Alliance, Chair: United Kingdom Sepsis Trust

Statement from Dr Dai Grove-White, Liverpool University

This report is a timely review of the current literature on the increasing problem of antimicrobial resistance (AMR) in human cases of *E. coli* infection in people and the possible association with antimicrobial usage in animals. It is particularly timely in the light of the “One Health” paradigm and the realisation that if the World is to feed itself then food production must increase exponentially over the next 50 years. The recent Foresight Report commissioned by the UK government refers to the concept of “sustainable intensification” which will certainly require judicious usage of antimicrobials. Agricultural use of antimicrobials is a key feature of intensive agriculture and essential both from an economic and welfare aspect. However antimicrobial usage in agriculture can be reduced considerably as is evident from the wide variation in usage seen between individual farms. The problem of animal derived AMR is best considered along with its “partners in crime” – foodborne zoonoses (FBZ) such as *Salmonella* and *Campylobacter*. Control of the human and animal disease burden associated with AMR and FBZ is possible but it has a cost which ultimately must be borne by the consumer. The current paradigm of “cheap food” actively works against this since disease control in intensive systems requires financial investment – for example in the dairy industry, considerable reduction in disease is possible by improving environmental conditions and nutrition but at the current time the return on investment is such that this is not always possible. Antimicrobial usage offers a “quick fix” to the problem although not a sustainable one. Recent research suggests that allowing broiler chickens to grow slower reduces *Campylobacter* colonisation and virulence but again this has a cost to the producer and ultimately the consumer.

There is currently considerable concern about the usage of 3rd and 4th generation cephalosporins and fluoroquinolones in agriculture due to an increasing evidence base suggesting agricultural usage has human health implications. It is almost inevitable that usage of these drugs will be restricted by law unless the industry can reduce significantly its usage.

The usage of these classes of drugs has risen considerably over the last few years and it is salutary to ask why? Due to the “cheap food” paradigm, there is little incentive for the animal health pharmaceutical industry to invest in new molecules or investigate new uses for existing drugs due to the very high costs of licensing products for food producing animals. Similarly, there is little incentive for costly well designed large scale clinical trials to provide high quality evidence on which the practising veterinarian can base decisions. The British Veterinary Association and other organisations have produced excellent guidelines on prescribing based on recognised good practice such as monitoring AMR profiles and using these in decision making. They advise that usage of 3rd and 4th generation cephalosporins and fluoroquinolones should be restricted only to cases where such evidence is available and no other drug class would be suitable. Alas, this is not the case in the real world – the chief driver for ceftiofur (a 3rd generation cephalosporin) usage in the dairy industry is that residues in milk do not exceed the permitted levels and therefore milk may be sold from animals under treatment. This is a perfectly rational decision for the farmer since the financial loss associated with discarding 5 – 7 days (length of treatment course & milk withdrawal period) worth of milk production (say 150 – 200 litres @ 28pence/litre) is considerable. With farmers barely being paid the production costs for their milk it may be unreasonable to ask them to forgo income on the grounds of “greater good”.

Similarly the pharmaceutical industry must recoup its considerable investments. This must generally be achieved within a relatively short time period before the patents expire after which the drug in question can be manufactured and sold as a generic product, invariably at a considerably lower price. Thus advertising to the farmer and veterinary surgeon are key tools used to increase sales and thereby recoup

the investments made. There have been efforts to ban advertising to the farmer but these have failed. It is of interest that advertising of medical drugs to the potential consumer is illegal in the EU although the pharmaceutical industry strives to bypass these restrictions. Banning of advertising to farmers would be a step in the right direction since it would reduce the commercial pressure on the veterinary surgeon to prescribe a particular drug which may not be warranted on purely clinical grounds.

The issue of AMR and agricultural usage urgently requires addressing but it cannot be considered in isolation and the debate must consider all aspects of farm animal production and in particular the price paid for the end product by the retailer and ultimately the consumer. It is essential that all the relevant stakeholders namely governments, farmers, veterinary surgeons, retailers and consumers participate in this debate to ensure the protection of both human and animal health and allow agriculture to rise to the inevitable challenges of the next 50 years without jeopardising human health.

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Executive summary

This report finds that the use of antibiotics in intensive livestock farming is contributing to one of the greatest challenges faced by modern medicine: the rise of antibiotic-resistant *E. coli* infections.

In 2003, *E. coli* overtook MRSA to become the leading cause of blood poisoning which kills an estimated 37,000 people a year in the UK. *E. coli* now cause more infections than any other disease-causing bacteria.

Some *E. coli* strains, such as the much-feared *E. coli* O157 cause food poisoning, however, this report is concerned with extra-intestinal pathogenic *E. coli* (ExPEC), which only cause infections outside the intestine. Like the food-poisoning strains, ExPEC can pass to us on food. In addition to blood poisoning, ExPEC cause three-quarters of all urinary-tract infections and a number of other infections, including meningitis in very young babies.

Many of these infections, including all blood poisoning, need to be treated with antibiotics to save lives, but levels of resistance have risen sharply over the past decade, increasing treatment failures.

Of particular concern is the rise in the incidence of highly resistant extended-spectrum beta-lactamase (ESBL) *E. coli*. Since 2003, a new type of ESBL *E. coli*, described by the Health Protection Agency as 'extremely resistant', has become much more common in the UK. These new ESBL *E. coli*, called CTX-M *E. coli*, show high levels of resistance to 'critically important antibiotics' and to most other antibiotic classes.

Unlike earlier types of ESBL *E. coli* which were restricted to hospitals, many CTX-M ESBL *E. coli* infections originate in the community. However, the antibiotics most strongly associated with the spread of ESBL resistance, the modern cephalosporins, are normally prescribed only by hospital doctors treating serious cases of infection, not by GPs seeing patients in their surgeries. Why then are so many of these ESBL *E. coli* infections originating outside hospitals, often in patients who have not been in a hospital for some time?

For the last few years this has been the underlying question which has prompted a global research effort. The use of antibiotics on intensive livestock farms and the associated spread of antibiotic resistance through the food chain has long been suspected as a cause, but some scientists, and in particular supporters of the intensive livestock industries, have been keen to play down any association with agriculture, and have highlighted confounding aspects of the research, and tried to focus attention instead on the part of this problem which is undoubtedly associated with the medical use of antibiotics.

This has led to regulatory inertia in the UK and in some other countries, and allowed large and continuing increases to occur in the farm use of the modern cephalosporins and other antibiotics associated with the spread of ESBL *E. coli*. As a result, ESBL *E. coli* has now become widespread on many cattle, pig and poultry farms in the UK. ESBL bacteria are also frequently found in food animals in countries to which many Britons travel for holidays, and from which meat is imported.

Evidence of the high levels of ESBL *E. coli* in British farm animals has come to light as a result of a substantial amount of research published by government scientists in the past year.

However, in spite of their important findings, with potentially serious consequences for human health, according to the minutes of a Defra scientific advisory committee, funding for future surveillance has

been cut: 'AHVLA told the meeting that as a result of the CSR [Comprehensive Spending Review], the surveillance budget had been reduced by 42% which had meant that ESBL work was currently unfunded'.

This report takes a detailed look at the evidence implicating the farm use of antibiotics, in large part the same research which caused one leading Danish government scientist to conclude that 'anyone still opposing a link between antibiotic use in food and animal production and its direct impact on human health does so for other reasons besides science'.

Main findings

Increasing incidence and antibiotic resistance of *E. coli* infections (see Chapters 2, 3 and 5)

Voluntary data collected from hospitals by the Health Protection Agency (HPA) show that for England, Wales and Northern Ireland, the number of reported *E. coli* blood-poisoning infections has increased nearly every year for the past two decades, from 7610 cases in 1990, to 11,369 in 2000, to 27,055 in 2010.

For the last seven months of 2011, the HPA has collected mandatory data on *E. coli* blood-poisoning infections from hospitals. Extrapolating from this data, and using data from a recent government report, Health Protection Scotland and scientific papers, the Soil Association estimates that in the UK in 2011 there were:

- 750,000 – 1,500,000 *E. coli* infections
- 60,000 ESBL *E. coli* infections
- 37,500 *E. coli* blood-poisoning infections of which 700 were in babies
- 3,000 ESBL blood-poisoning infections
- 7,700 deaths from *E. coli* infections
- 1,500 deaths from ESBL *E. coli* infections.

The rapid rise of *E. coli* blood poisoning over the past decade has come at the same time as a dramatic increase in the level of resistance to key antibiotics commonly used to treat these infections:

Resistance to key antibiotics used to treat *E. coli* blood-poisoning infections

	1990	2000	2010 UK average	Some hospitals 2010
Modern cephalosporins	1%	2%	10%	23.6%
Fluoroquinolones	1%	4%	19%	38.5%
Gentamicin	2%	3%	9%	14.3%

Resistance in *E. coli* urinary-tract infections has also reached extremely high levels in the UK, and some antibiotics formerly prescribed by doctors are no longer suitable for routine treatment. Scientists believe that more urinary-tract infections are now developing into blood poisoning because antibiotics have failed to clear the infection, which in turn is increasing the number of blood-poisoning infections.

The number of ESBL *E. coli* blood-poisoning infections is also increasing sharply: in 2000 there were approximately 200 ESBL blood-poisoning infections, but by 2011 this had increased to approximately 3,000.

Patients with *E. coli* blood poisoning are almost 3 times more likely to die if they have ESBL-resistant *E. coli*.

The HPA said in 2005 that, if it is found to be commonplace that people in the community carry ESBL bacteria in their intestines, then 'this may point towards the food chain being a potential source'. Since then, there has been a large increase in the gut carriage of these bacteria: in 2005, between 0.25% and 1.4% of hospital and community patients had ESBL *E. coli* in their faeces, but a study carried out in Birmingham last year looked only at community patients and found that 11.3% of them had ESBL *E. coli* in their faecal matter.

Elderly patients are at present those who are most at risk from ESBL *E. coli* infections in the UK. However, Health Protection Agency scientists are concerned that this may change over time, as it already appears to have done in some other countries, and more infections will occur in younger people. They warn that rising rates of ESBL *E. coli* in the genitourinary tracts of sexually active women raise the 'alarming possibility' that the resistance could be transferred to sexually transmitted pathogens, such as gonorrhea. Because modern cephalosporins are such important antibiotics for treating gonorrhea, they say that this would be 'a catastrophic development'.

Emergence of ESBL *E. coli* in British farm animals (see Chapter 6)

Defra claimed in 2006, before any active surveillance had been carried out, that ESBL *E. coli* was at 'a very low level in livestock'. However, recent Defra surveys have found ESBL *E. coli* on:

- 18 of 48 (37.5%) cattle farms
- 12 of 23 (52%) of poultry abattoirs and 3.6% of individual birds
- seven of seven pig farms (six of which were linked as a network of farms) and from 438 of 504 (86.9%) of pigs
- boot swabs from 18 of 337 (5.3%) turkey farms (the birds were not tested).

Veterinary Laboratory Agency (VLA) scientists have warned that animals which are 'high-density shedders' of ESBL *E. coli* may pose a greater risk of contamination of carcasses going into the food chain when the animals go to slaughter. Of the animals which had ESBL *E. coli* in their faeces, they found that 46.9% (15 of 32) chickens, 40% (8 of 20) of pigs and 8.6% (3 of 35) of cattle were high-density shedders.

ESBL *E. coli* on food (see Chapter 6)

There is no information on the current level of ESBL *E. coli* on home-produced retail chicken or any other British meat. Only one study has tested UK-produced raw retail meat. This was carried out in 2006, and one of 62 chickens tested was contaminated with ESBL *E. coli*. Imported poultry meat had much higher levels: nine of 27 (33.3%) samples of imported chicken and seven of 40 (17.5%) samples of chicken of unknown origin were also positive.

As levels of ESBL *E. coli* in farm animals have increased greatly since 2006, we summarise studies from other European countries to give an indication of possible levels of contamination in the UK.

Percentage of retail meat samples which were positive for ESBL *E. coli* , with number of positives and number of samples in brackets

Country	Year published	Chicken	Pork	Beef	Other
UK (home produced)	2008 (sampled in 2006)	1.6% (1/62)	-	-	-
UK (imported)	2008 & 2010	30% (71/237)	-	-	-
UK (unknown)	2008	17.5% (7/40)	-	-	-
Netherlands*	2011	100% (1/1)	0% (16)	5.9% (17)	-
Netherlands	2011	77% (68/80)	2% (1/57)	5% (5/85)	-
Netherlands	2012	100% (92/98)†	-	-	-
Denmark	2011	3.3% (4/121)	2% (3/153)	0.7% (1/142)	-
Spain	2010	67% (8/12)	25% (3/12)	8% (1/12)	58% (7/12)a
Spain	2008	57% (27/47)	0% (0/30)	0% (0/22)	58% (7/12)b
France*	2011	43% (15/35)	0% (0/1)	-	-
Germany*	2011	34% (50/149)	0.7% (1/142)	0% (0/27)	-

*Tested as imported meat in Denmark; a Turkey; b Rabbit; † included 32 of 38 organic chickens

One study in the Netherlands tested for ESBL *E. coli* in organic chicken, and found that 84% were positive. The number of ESBL bacteria per positive sample was four times lower than for non-organic chickens. It is believed that the main reason for the high levels in organic chicken is that many Dutch organic poultry farmers buy in one-day-old chicks from conventional producers. According to Soil Association organic standards, producers may only buy in non-organic livestock if organic livestock, or in conversion livestock, are not available, and then only with the Soil Association's approval. In 2011 approximately 80% of organic chickens certified by the Soil Association came from organic hatcheries, and the remaining 20% came from a non-organic hatchery.

Scientists find increasing evidence that farm animals are an important source of resistance in *E. coli* (see Chapters 4 and 8)

Antibiotic resistance can be transmitted from farm animals to humans in three main ways:

- through the food chain (this is the most common way)
- through the environmental when untreated manures are spread on the land
- by direct contact with farm animals.

Resistant *E. coli* from farm-animals can colonise the intestines, then cause infection at a later date. *E. coli* from farm animals can also transfer resistance genes to human *E. coli* inside the intestines.

In both cases the *E. coli* may not cause infection until much later. Scientists are now finding strong evidence that a significant amount of resistance in human *E. coli* infections comes from farm animals, contributing to increasing resistance in urinary-tract infections and blood poisoning.

Research published in December 2011, based on data collected from 11 European countries (not including the UK), found that the rates of resistance in *E. coli* causing blood-poisoning in humans were strongly correlated with the rates of resistance of farm-animal *E. coli*, particularly for poultry, but also for pigs and cattle. The authors concluded that 'a large proportion of resistant *E. coli* isolates causing blood-stream infections in people are likely to be derived from food animal sources'.

British and other scientists have found very strong evidence that resistance in human *E. coli* emerged after the use of certain antibiotics in agriculture. This has had consequences for human treatment, since

the resistances which emerged also made the bacteria resistant to antibiotics that were used in human medicine. One scientific review found that ‘these observations strongly indicate that resistance to streptothricin and apramycin emerged primarily among food animals because of the selection by the use of these antibiotics for food animals and that, subsequently, resistant bacteria were transmitted to humans’.

Chicken meat is considered to be a key source of human resistant *E. coli*. Several studies have found that antibiotic-resistant *E. coli* from humans are more genetically similar to both antibiotic-resistant and antibiotic-sensitive *E. coli* from chicken than to antibiotic-sensitive *E. coli* from humans. The antibiotic-resistant and antibiotic-sensitive *E. coli* from chicken were found to be very closely related. This suggests that the antibiotic-resistant *E. coli* in humans may have emerged in poultry and then been transmitted to humans, probably via the food chain.

In one study, six volunteers ate a near-sterile diet for an average of 17 days after a prior control period of three weeks during which they had eaten their normal diet. The *E. coli* in their faeces were monitored during both periods. Once the sterile diet began, the number of antibiotic-resistant *E. coli* in the volunteers’ faecal matter fell significantly, whereas the number of sensitive *E. coli* did not fall significantly. This suggests that food is a significant source of resistant *E. coli*.

Outbreaks of urinary-tract infections caused by a single *E. coli* strain have occurred over large geographical areas in unrelated people. This has led many scientists to suspect a foodborne source of some infectious *E. coli*.

Subsequent molecular studies comparing *E. coli* from retail chicken with *E. coli* causing urinary-tract infections in humans have found ‘strong support for the role of food reservoirs or foodborne transmission in the dissemination of *E. coli* causing common community-acquired urinary-tract infections’. Not all animal *E. coli* are suspected of causing these infections, but numerous studies from around the world have now found that some farm-animal *E. coli* strains can cause urinary-tract infections. Some of the strongest evidence has come from a recent series of studies by Danish government scientists, which has found ‘solid evidence’ that some urinary-tract infections in humans are caused by farm-animal *E. coli*.

Research carried out in the UK and published in 2010, has found that when animal *E. coli* acquire antibiotic resistance this may increase the likelihood the bacteria will be transmitted to humans: they found multiple antibiotic-resistant *E. coli* were better able to withstand the slaughter and chilling process than the sensitive *E. coli*. They said this ‘increased the likelihood of them passing along the food chain [to humans]’.

One scientific review has concluded that there is now ‘accumulating data that support the likelihood that animal reservoirs could be responsible for contamination of humans with antimicrobial- resistant ExPEC [extraintestinal pathogenic *E. coli*] and other bacteria through the consumption of contaminated food’.

Some human ESBL resistance is of farm-animal origin (see Chapter 7)

ESBL resistance genes are carried on small pieces of DNA called plasmids, which are separate from the *E. coli*’s chromosome. These plasmids, and the resistance genes they carry, can replicate inside the *E. coli* and be transmitted to other *E. coli* strains, thus spreading the resistance. This can happen inside the human gut. So farm-animals can be a source of ESBL *E. coli*, but also of ESBL resistance genes.

Defra and HPA scientists acknowledge that the ‘emergence of ESBL bacteria in food producing animals may present a risk of resistant strains being transmitted to humans through the food chain’.

Although the medical use of antibiotics clearly contributes to the frequency of human ESBL resistance, British studies have found significant evidence of ESBL resistance genes being transmitted between farm animals and humans. One VLA study of an ESBL plasmid commonly found in humans also found an indistinguishable ESBL plasmid in cattle. The scientists believed that the plasmid had spread from humans to cattle, but this nevertheless provides evidence that it could also pass in the other direction.

Scientists from Birmingham studied another ESBL plasmid and found the same plasmid *E. coli* from cattle and in ESBL *E. coli* causing human clinical cases in the UK. They said this showed the plasmid could transfer between animal and human *E. coli*.

The ESBL types which are most common in human clinical infections in the UK are CTX-M-15 and CTX-M-14. These are also the two most common ESBL types found in British cattle and in British turkey. Furthermore CTX-M-15 is the second most common CTX-M type in British poultry and CTX-M-14 has also been found in British poultry. These two CTX-M types have not yet been found in pigs, but only five pig isolates have been tested in the UK.

Most of the ESBL *E. coli* strains found in British chicken and turkey are known to have caused ESBL *E. coli* infections in humans in the UK or abroad, so these bacteria clearly have the potential to infect humans.

In the UK, and in some other countries, there is one dominant strain of ESBL *E. coli* in human medicine, called ST131, which carries CTX-M-15 resistance. The ST131 strain has recently been found in one case in British cattle with ESBL resistance, most likely CTX-M-15 resistance, but minor differences were found with the most prevalent human clones. It appears, therefore, that this strain is primarily circulating amongst humans, but a farm-animal link cannot yet be ruled out. However, the dominance of this strain is no longer as strong as it was as it was, and now it accounts for only 45% of human ESBL *E. coli* in the UK. A greater diversity of strains and of ESBL genes is emerging, and provides circumstantial evidence of an increasing farm-animal link.

In countries, such as the Netherlands, where the ESBL *E. coli* epidemic in farm animals is at a more advanced stage than it is in the UK, and where more research, particularly in retail meat, has been carried out, there is now very strong evidence that farm animals are important reservoirs of human ESBL *E. coli*, or of their resistance genes.

Dutch scientists have found that 35% of human clinical cases of ESBL *E. coli* have ESBL resistance genes which are genetically indistinguishable from those found in poultry and in chicken meat. The scientists, including government scientists have said that 'These findings are suggestive for transmission of ESBL producing *E. coli* from poultry to humans, most likely through the food chain'. One Dutch government scientist has said the evidence 'strongly suggests that poultry products are the source for humans [of ESBL *E. coli*]'.

The European Food Safety Authority has said the genetic similarities between certain ESBL plasmids found in farm animals and in humans 'strongly suggests an animal reservoir for this ESBL gene variant'.

Recent Japanese research has shown that all the ESBL resistance genes found in *E. coli* in live poultry had been found in human clinical cases in that country. Italian research has found that some ESBL *E. coli* from poultry are likely to be able to colonise humans, and transfer their resistance genes to other pathogenic *E. coli* in the human intestine.

Studies have also shown that Danish pig farmers and Dutch poultry farmers are much more likely than the general population to carry ESBL *E. coli* in their intestines. Furthermore, the farmers frequently

carried the same type of ESBL resistance as their animals, but in different *E. coli* strains, which is evidence that genes are transferring rapidly between bacteria.

Several Spanish studies of gastroenteritis outbreaks have found an ESBL *E. coli* in a significant number of the affected people. In many of the outbreaks, the same strain of ESBL *E. coli* was found in more than one person. These may not have been ExPEC strains of *E. coli*, but the studies provide evidence that the ESBL resistance could be foodborne.

Farm antibiotic use is increasing the risk of *E. coli* being transmitted to humans (see Chapter 8)

American research published in January of this year found that adding certain antibiotics to pig feed increased the total number of *E. coli* bacteria in pig faeces 20 to 100 fold. Their research confirms the findings of a small number of studies carried out in the 1950s, 1970s and 1980s which had found similar results.

It is believed that certain antibiotics, which are inactive against all *E. coli* bacteria, can kill other bacteria in the animals' intestines, leaving more room and nutrients for *E. coli* to grow. VLA scientists, who found evidence of a similar effect in research published in 2006, suggested this might be happening.

The use of antibiotics, which did originally kill *E. coli* bacteria, can also favour their growth in animals' intestines once a high percentage of the *E. coli* have developed resistance.

Higher numbers of *E. coli* in animals' intestines are likely to increase the contamination of carcasses at slaughter, which in turn increases the chance of the bacteria reaching humans.

Farm antibiotic use per animal is increasing (see Chapter 9)

In 2000, the then government made a commitment to develop 'a coherent strategy aimed at reducing the veterinary use of antibiotics' because of increasing concerns about antibiotic resistance being transferred from farm animals to humans.

Although the total amount of antibiotics used on farms has fallen over the past decade, it has not fallen as fast as animal numbers and in particular not as fast as pig numbers (pigs account for approximately 60% of all farm antibiotic use).

According to Soil Association calculations, once the fall in animal numbers in each species is taken into account, the rate of antibiotic consumption has actually gone up by 18% between 2000 and 2010. In 2010, the rate of consumption per animal reached its highest ever level (statistics are only available from 1998 onwards).

Of particular concern is the increased use of two families of antibiotics, classified as critically important in human medicine by the World Health Organization, which are strongly linked with increasing levels of ESBL *E. coli*:

- modern cephalosporins use has increased in nine of the last ten years and by sixfold in total
- fluoroquinolones use has increased in seven of the last ten years, and by over 80% in total.

In contrast, in hospitals the use of modern cephalosporins and fluoroquinolones has been reduced by one third over the past five years.

The UK remains the only EU member state which continues to permit the advertising of antibiotics directly to farmers. In contrast to the UK, several other European countries are currently setting targets and taking action to reduce farm antibiotic use.

The emergence of ESBL *E. coli* in poultry in the UK and throughout Europe is believed to be due to the 'off-label' use of modern cephalosporins. Modern cephalosporins are not licensed for use in poultry, but vets can prescribe antibiotics which are licensed for use in other species in exceptional one-off situations. However, in many cases one-day-old chicks have been routinely injected with the antibiotics, which is not a permitted form of off-label use and therefore illegal.

Approximately 60% of UK farm antibiotic use is in pigs, 36% in poultry, 4% in cattle, and less than 0.5% in sheep. This is reflected in the levels of resistance in each species. A study co-authored by Defra scientists, found that 92.1% of *E. coli* from pigs, 5.7% of *E. coli* from cattle and just 3% of *E. coli* from sheep were resistant to at least one antibiotic (the study did not include poultry).

Defra research has shown that antibiotic use in UK organic pig and poultry production is much lower than in non-organic production. A survey of five organic and seven non-organic pig farms showed that the non-organic farms used between 13 and 330 times more antibiotic per kilo of meat produced than the highest-consuming organic farm. Only one of seven organic poultry farms surveyed used any antibiotics during the two years of the study, and this was only on one occasion. In contrast, in the Netherlands non-organic chickens get on average four courses of antibiotics in their short 42-day lives. The same situation is likely to be occurring in the UK. Published information is not available, but an industry source has told the Soil Association that almost all farm assured non-organic chickens are put on routine prophylactic antibiotics the day they are hatched.

The much lower level of antibiotic use on organic farms is reflected in much lower levels of resistance. Defra research found that the median number of antibiotics to which the *E. coli* from the organic poultry farms were resistant was just one, whereas for the non-organic poultry farms it was five. Research funded by the Scottish Executive also found much lower levels of resistance in *E. coli* from organic pigs compared with non-organic pigs.

Very low concentrations of antibiotics can select for resistance in *E. coli* (see Chapter 10)

Recent research by Swedish scientists found that extremely low concentrations of antibiotics can select for antibiotic-resistant *E. coli*. This confirms the findings of earlier work by British scientists who had found a similar effect.

The concentrations at which this effect occurs for some antibiotics were well below the maximum residues permitted in food. This suggests that legal residues in food could be having a selective effect in the human intestine, favouring resistant *E. coli* over sensitive *E. coli*. This would then make any subsequent *E. coli* infection more likely to be antibiotic resistant.

Recommendations

Recommendations to the Government and retailers

1. The UK's regulatory system for farm antibiotics was designed to limit the level of antibiotic residues in food and needs significant upgrading to address the issue of antimicrobial resistance as well. We recommend that the Government establish the factors that lead to the development of resistant strains of bacteria in farm animals and in the food chain, consider the approaches adopted in other EU countries and draw up a blue print for an improved regulatory system that is appropriate for addressing the farming dimension of one of the key emerging health concerns of the 21st Century.
2. The Government should take back control of policy work to address the use of antibiotic resistance in farm animals and the food chain. It was not appropriate to hand this responsibility, as the Government did last year, to the Veterinary Medicines Directorate, an executive agency which is largely funded by the pharmaceutical and farming industries.
3. The Government should set a target to halve the overall use of antibiotics on farms within five years, and develop policies to ensure the target is met. There should be enhanced monitoring and greater transparency of veterinary prescribing and farm use of antibiotics.
4. The Government should actively support proposals currently under discussion by the European Commission to phase out the preventative use of antibiotics in groups of healthy animals and prohibit all off-label use of modern cephalosporins and fluoroquinolone antibiotics.
5. Leading retailers should ensure that the farms that supply them phase out the preventative use of antibiotics in groups of healthy animals and do not use modern cephalosporins and fluoroquinolone antibiotics off-label or as first line treatments.
6. The Government should explore the possibility of encouraging farming systems with low use of antibiotics per tonne of meat, litre of milk etc. or dozen eggs, through EU farm payments.
7. The Government must make sure there are adequate funds for the Food Standards Agency to undertake comprehensive testing to establish the levels of ESBL *E. coli* on retail food in the UK.
8. The Government should ensure there are adequate funds for the Animal Health and Veterinary Laboratories Agency to increase its monitoring of ESBL *E. coli* of farms and also maintain an adequate level of research in this area. Defra's budget for this work should be considered in the context of the potential costs to the NHS if the problem of ESBL *E. coli* is allowed to escalate further and, in particular, if it becomes widely established in *Salmonella* as well.
9. The Government should work constructively at a European level to define more precisely the circumstances under which antibiotics can be used on a herd, flock or group basis.
10. If the use of modern cephalosporins and fluoroquinolones cannot be greatly reduced by voluntary measures, the farm use of modern cephalosporins should be banned and the use of fluoroquinolones restricted to mammals in life-saving situations.
11. The UK should immediately prohibit the advertising of antibiotics to farmers. Advertisements to veterinary surgeons should be purely factual and not emotive in any way.
12. To prevent the development of ESBL *E. coli* in calves, current guidelines discouraging the use of milk containing antibiotic residues for the feeding of calves or other livestock should be given legislative force.
13. The Veterinary Medicines Directorate's Inspections Administration Team should be given additional powers and training to undertake more thorough inspection of livestock farms which

are permitted to incorporate antibiotics into feed. This should include unannounced visits and feed sampling to check that inclusion rates are not below full therapeutic levels.

14. All farmers should be required to compost farmyard manure thoroughly in order to kill off *E. coli* bacteria. Livestock slurry should be thoroughly aerated before spreading.

Recommendations to the farming and veterinary industries

15. The British Poultry Council's voluntary initiative to stop using cephalosporin antibiotics and to reduce the use of fluoroquinolones in poultry production is to be welcomed. Similar moves by other sections of the livestock industry should be encouraged.
16. The British Veterinary Association (BVA) should make renewed efforts to draw its 8-point plan to the attention of all veterinary surgeons. This provides excellent guidance on the prescribing of antibiotics and recommends using modern cephalosporins and fluoroquinolones in limited situations only.
17. Veterinary surgeons should agree not to prescribe modern cephalosporins for dry-cow therapy or for use in suckling cows, in order to prevent calves ingesting milk containing their residues.
18. The BVA, Royal College of Veterinary Surgeons and the Government should consider how veterinary practices could best submit returns detailing the antimicrobials they have prescribed and the reasons they were needed. Results should then be analysed and summaries published annually, showing the key reasons for usage and how much of each antibiotic class was used in each species.

1. Introduction

Blood poisoning (also known as sepsis) kills 37,000 people every year in the UK. That is more than breast, bowel and prostate cancer combined. The UK Sepsis Trust is calling for it to be made a medical emergency and a clinical priority for the NHS [1]. The biggest single cause of blood poisoning in Britain is now *Escherichia coli* (*E. coli*), which has overtaken *Staphylococcus aureus* (including MRSA) since 2003 [2].

E. coli have been called 'the most important human pathogen on a global scale' [3]. In 2003 it was estimated that they cause up to 175 million infections worldwide every year [4] and, according to Dr David Livermore from the Health Protection Agency, they are now 'turning nasty' [5].

Most people have heard of *E. coli* O157 which causes food poisoning, and many people will know about the outbreak of food poisoning caused by the related *E. coli* O104 in Germany in June 2011. These are part of a large group of *E. coli* that cause diarrhoea, which is sometimes very severe due to the release of toxins [6]. It is well known that some of these can pass from farm animals to humans on food, both meat and vegetables. David Livermore's comment, however, does not relate to this type of *E. coli*.

There are two other major families: commensal *E. coli* and extra-intestinal pathogenic *E. coli* (ExPEC). Both of these are harmless while they remain in the gut, but ExPEC cause a range of infections elsewhere in the body. They are the biggest single cause of blood poisoning, urinary-tract infections and infectious disease in humans in the UK.

Pathogenic *E. coli* of this sort also cause serious disease in farm animals. Large numbers of chickens die every year from *E. coli* infections; *E. coli* mastitis in dairy cows can be very severe and lead to the death of the cow, and adult pigs and cattle can be affected by urinary-tract and other infections caused by these pathogenic *E. coli* [6].

ExPEC are now causing more infections than ever before, but the reason they have been described as 'turning nasty' is because *E. coli* blood poisoning in particular is becoming much harder to treat than it used to be, due to rising levels of antibiotic resistance. Over the last decade, levels of resistance to the two main antibiotic classes previously used to treat life-threatening *E. coli* infections have increased dramatically [5]. The situation is not just confined to the UK. In most other European countries there are similar trends; in Southern and European countries they are even higher, while in some countries such as India, the situation is more serious still [7].

Trying to establish what is causing these rapid increases in antibiotic resistance is now the subject of a major worldwide research effort. What makes the problem so urgent is that there is little prospect in the next few years of suitable new antibiotics becoming available for treating *E. coli* infections that might fail to respond to existing treatments [5].

One of the main concerns is the emergence of a new type of antibiotic resistance, called extended-spectrum beta-lactamase *E. coli*, or ESBL *E. coli*. This first emerged in 1983 [8], but remained extremely rare until a decade ago, when new, even more resistant variants started to emerge. ESBLs are enzymes produced by bacteria that cause resistance to modern cephalosporins¹, antibiotics classified by the WHO as 'critically important in human medicine' which had become the treatment of choice for serious *E. coli*

¹ Modern cephalosporins are the 3rd and 4th generation cephalosporins.

infections. According to the Health Protection Agency, modern cephalosporins are ‘workhorse hospital antibiotics, given as first-line agents to many severely-ill patients’ [9]. Because of high levels of resistance to other important antibiotics, hospital doctors are increasingly having to use the most powerful antibiotics still available, the carbapenems, which is building up further problems for the future by speeding up the development of resistance to these as well.

The new ESBL *E. coli*, called CTX-M ESBL *E. coli*, are more virulent and have rapidly become the most common form of ESBL *E. coli* in the UK and in Europe [10]. Unlike earlier types of ESBL *E. coli* which were almost exclusively restricted to hospitals [11], CTX-M ESBL *E. coli* mainly affect community patients [10]. However, modern cephalosporins, the antibiotics which most strongly select for and promote the spread of ESBL *E. coli*, are usually only prescribed by hospital doctors treating serious cases of infection, not by GPs seeing patients in their surgeries (because these antibiotics mostly have to be injected into a vein or muscle). Why then are so many of these ESBL *E. coli* infections originating outside hospitals, often in patients who have not been in a hospital for some time?

One major area of investigation is the contribution to the problem being made by intensively farmed animals. Due to the high level of antibiotics still used in livestock production, strains of *E. coli* in farm animals have become increasingly resistant to antibiotics too. In particular, the farm use of modern cephalosporins has increased dramatically over the last decade, and as a result ESBL *E. coli* have increasingly been found on cattle, pig and poultry farms.

Establishing just where particular strains of *E. coli* that cause infections in humans originate is extremely difficult. In part, this is because we all have a resident *E. coli* population in our intestines, and the strains can vary significantly from one person to another. In some people it seems these resident strains are stable, while in others they can change, partly at least due to the ingestion of *E. coli* on food [12].

It has also been known since the 1960s that farm-animal strains of *E. coli* can transfer their antibiotic resistance to resident human *E. coli* in the human gut² [13]. This means that even when ingested farm-animal *E. coli* do not become established in the intestines or go on to cause infection directly, they may nevertheless survive in the gut for long enough to pass on their resistance genes to the resident *E. coli* bacteria. More recent research has shown that, in addition to antibiotic-resistance genes, a wide range of virulence factors, which make it possible for pathogenic *E. coli* to cause infection, can also be passed from one strain of *E. coli* to another [8]. In this respect, the gut is a vast mixing bowl where large numbers of different types of *E. coli* from different sources, some harmless, some pathogenic, some carrying antibiotic resistance, some carrying virulence factors, interchange genetic material with each other, and with resident *E. coli* on a continual basis.

Adding one further twist, there is evidence that antibiotic-resistant *E. coli* from farm animals could be passing to humans from the spreading of untreated animal manures on farmland, and quite possibly also from humans back to farm animals through the spreading of inadequately treated human sewage on farmland [10]. The relative significance of these two factors has not been carefully assessed but it is of note that most human sewage is treated before being spread on farmland, while most intensively produced animal manures are not³.

² ‘Horizontal gene transfer’ between bacteria occurs when donor bacteria, carrying a particular gene, make more copies of the gene and transfer them to other bacteria. If the gene is, for example, an antibiotic-resistance gene, the receiving bacteria become antibiotic-resistant.

³ Soil Association organic food and farming standards require any manure brought onto an organic farm to be properly composted, a key aim of which is to kill pathogenic *E. coli* bacteria.

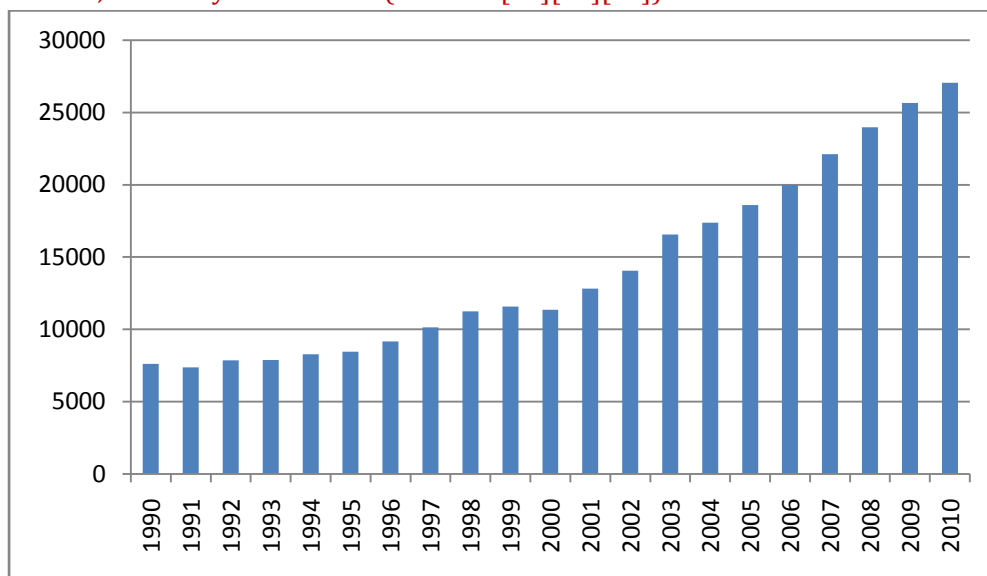
As a result of these multiple factors and uncertainties, it is remarkable how much progress has already been made in unraveling the situation and monitoring it as it progressively changes. This report reviews a large number of scientific studies in order to address two questions:

1. Is the overuse of antibiotics in agriculture contributing to the problem of antibiotic resistance in *E. coli* infections in humans?
2. Could it also be contributing to the large increase in the number of *E. coli* infections as well?

2. Increasing incidence of *E. coli*

No reliable statistics are available for the number of urinary-tract infections in the UK, but it is known that about 70-80% of all UTIs are caused by *E. coli* [14]. For blood-poisoning infections, statistics have been collected, under a scheme where reporting was voluntary, first by the Public Health Laboratory Service and more recently by the Health Protection Agency (HPA). They show that these infections have been increasing since at least 1990. Over the last decade, the growth in such infections has accelerated significantly. Graph 1 shows that there were 7,610 blood poisoning infections reported in 1990, by 2000 this had increased to 11,369 and by 2010 to 27,055 [15][16][17]. *E. coli* is now by far the most important cause of blood poisoning in the UK [2].

Graph 1 Number of *E. coli* blood-poisoning infections in England, Northern Ireland and Wales, 1990 to 2010, voluntary surveillance (sources: [15][16][17])



2.1. Estimate of the number of *E. coli* infections, blood-stream infections, ESBL *E. coli* infections and deaths in 2011

In June 2011, mandatory surveillance for *E. coli* blood-poisoning infections was introduced for England, and in the first seven months a total of 18,924 infections were recorded, an average of just over 2,700 a month [18]. If we conservatively estimate that the number of infections averaged 2,600 per month for the first five months of the year, this means that in England alone there were actually 32,000 *E. coli* blood-poisoning infections in 2011, almost 5,000 more cases than indicated by voluntary reporting for England, Wales and Northern Ireland in 2010.

In Scotland, the introduction of electronic reporting in 2008 led to a large increase in reported cases of *E. coli* blood infections to 3,602 in 2010 [19]. If we assume a similar number occurred in 2011 (a conservative assumption, as the number has increased each year since electronic reporting was introduced), then the total number of infections for England and Scotland in 2011 was approximately 35,600. Assuming that Wales and Northern Ireland had similar infection rates, we can estimate, using population data, that in 2011 there were approximately 38,500 *E. coli* blood-poisoning infections in the UK.

The rate of resistance to modern cephalosporins has been reported at 10% for 2009 and 2010. If we assume a similar rate for 2011, then approximately 3,850 blood-poisoning infections were resistant to modern cephalosporins. According to the HPA, BSAC surveys have found that 78-90% of resistance to modern cephalosporins is due to ESBL resistance [20][21]. If we assume that 80% of modern cephalosporin resistance is due to ESBLs, that equates to 8% ESBL resistance in blood-poisoning infections in 2011. This means there were approximately 3,000 ESBL *E. coli* blood-poisoning infections in 2011.

A recent ESBL report by the government's advisory committees DARC (Defra Antimicrobial Resistance Coordination Group) and ARHAI (Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection) said that blood-poisoning only accounts for approximately one in 20 ESBL infections [10]. This means that in 2011 there were very approximately 60,000 ESBL infections. From this figure, we can calculate a rough estimate of the total number of *E. coli* infections in the UK in 2011: if ESBL resistance in all infections was at 8%, then the total number of infections was approximately 750,000.

In reality, according to one British study, it appears that ESBL resistance in urinary-tract infections is at about half the level of the resistance in blood-poisoning infections [11], which would mean about 4% in 2011. The authors say 'This may reflect inadequate treatment of those urinary tract infections where an ESBL-producer is present, leading to an "overspill" bacteraemia' [11]. If ESBL resistance in all *E. coli* infections was just 4%, the total number of *E. coli* infections in 2011 could have been as high as 1.5 million.

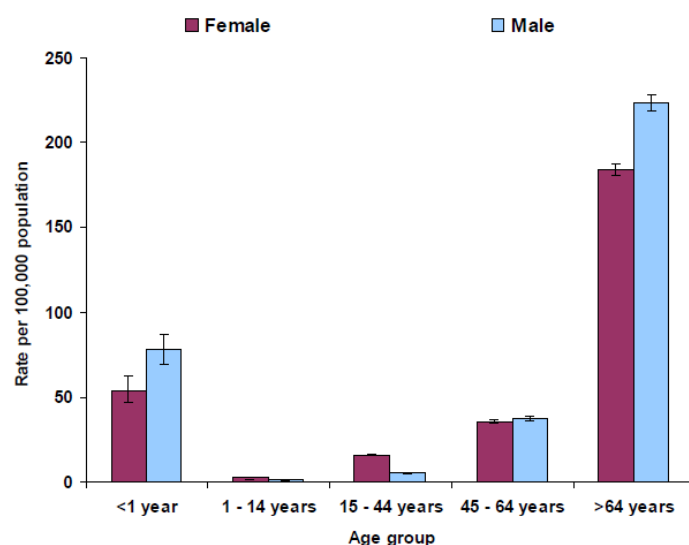
Since one in five people with *E. coli* blood-poisoning infections die [22], we can estimate that in 2011 there were 7,700 deaths caused by *E. coli* blood poisoning. The death rate from ESBL *E. coli* blood-poisoning has recently been estimated to be about 2.9 times higher than for non-ESBL blood poisoning [23], so we conservatively estimate there were approximately 1,500 deaths from ESBL *E. coli* in 2011. Table 1 summarises all of these estimates.

Table 1 Soil Association estimates on *E. coli* infection incidence in the UK in 2011

Total number of infections	750,000 – 1,500,000
Blood-poisoning infections	38,500
Total number of ESBL infections	60,000
ESBL blood-poisoning infections	3,000
Deaths from blood poisoning	7,700
Deaths from ESBL blood poisoning	1,500

The Health Protection Agency also provides data on the age and sex distribution of those affected by *E. coli* blood-poisoning. This shows that those most at risk are those aged 65 and over and babies aged under one year of age [24]. See Graph 2.

Graph 2 *E. coli* blood-poisoning in 2010 in England, Wales and Northern Ireland by age and sex, based on voluntary reporting (source for graph: HPA [24])



We can now estimate the approximate number of *E. coli* blood-poisoning infections by age group, using the above data, and adjusting for the fact that mandatory data collected in England from June 2011 onwards shows that, according to our estimates, the number of *E. coli* blood-poisoning infections in England, Wales and Northern Ireland in 2011 was about 29% higher than the number of infections reported by voluntary data in 2010. Our estimates for the number of blood-poisoning infections by age group, using population data from the Office for National Statistics [25], are given in Table 2, and show that approximately 700 babies developed *E. coli* blood poisoning in 2011.

Table 2 Soil Association estimate for the number of *E. coli* blood-poisoning infections in 2011 by age group

< 1 year	1-64 years	65 years and over
700	9,800	28,000

3. Increasing antibiotic resistance of *E. coli*

E. coli food poisoning and 'haemolytic-uremic syndrome', caused by the notorious O157 or other shiga-toxin-producing strains, can be very serious infections. However, antibiotic therapy is not usually recommended as it can cause increased toxin production by the bacteria. As a result, trends in the level of antibiotic resistance in *E. coli* which cause food poisoning are not normally of great importance in relation to the treatment of these infections.

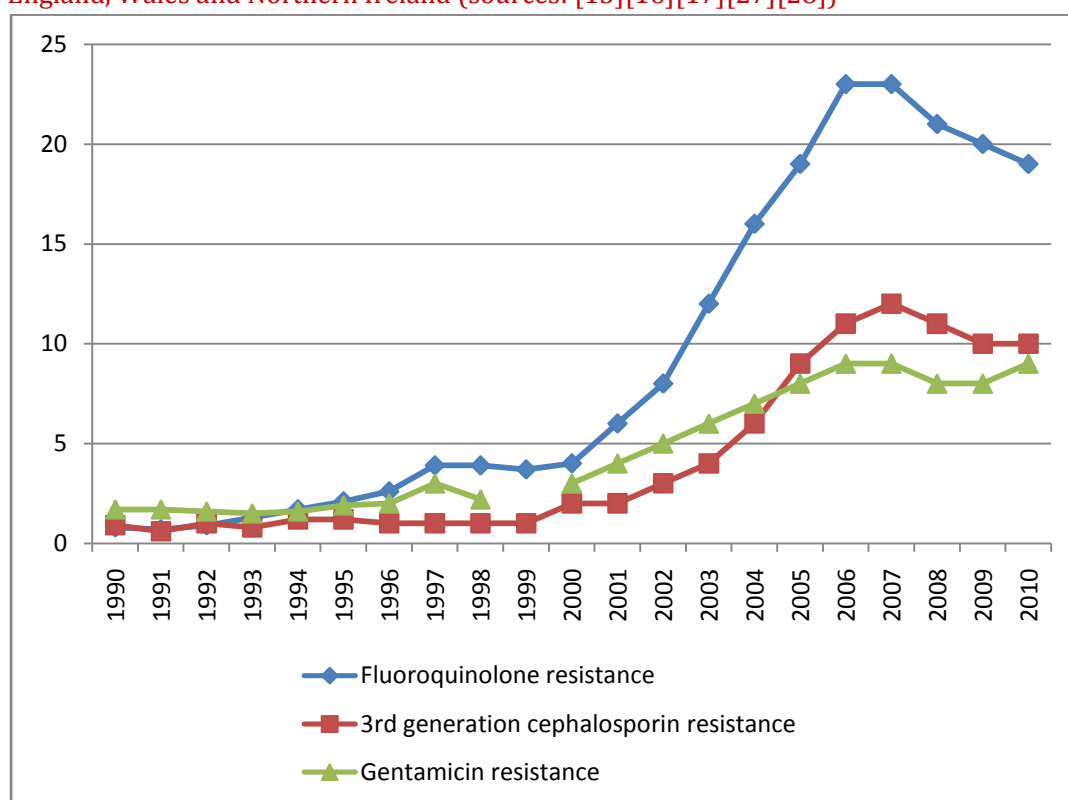
On the other hand, the *E. coli* which cause extraintestinal pathogenic (ExPEC) infections, infections outside the gut, are a different type of *E. coli*, and can be treated with antibiotics. These infections, include mainly urinary-tract infections (infections of the bladder and possibly kidneys) and blood poisoning, but also meningitis in newborn babies, surgical peritonitis, skin and soft-tissue infections, and more rarely infections of the pancreas and gallbladder. The level of antibiotic resistance in these *E. coli* is therefore very important in relation to treatment. This is particularly significant since there has been an increase the level of resistance to the antibiotics most widely used for treatment, in addition to the increase in the number of *E. coli* infections over the past decade.

Data collected by the former Public Health Laboratory Service and by the Health Protection Agency show that resistance in *E. coli* blood-poisoning infections to some of the antibiotics most widely prescribed by hospital doctors for treating serious cases has been increasing for twenty years [15][16][17].

In the last decade, the rates of resistance to 3rd generation cephalosporins and fluoroquinolones, two of the most important classes of antibiotic that have been kept in reserve for treating *E. coli* infections that fail to respond to initial and second-line courses of antibiotics, have increased sharply. Both of these are classified as 'critically important in human medicine' by the WHO. The Health Protection Agency warned in 2005 'Thus, two major drug classes are being undermined concurrently' [26]. Rates of resistance to gentamicin, another important antibiotic used to treat complicated urinary-tract infections or *E. coli* infections in newborn babies, have also increased sharply. See Graph 3 [15][16][17][27][17][28].

Referring to *E. coli*, Dr Livermore from the HPA has said, 'Since about the turn of the century it's been getting, in bacteraemias [blood-stream infections], dramatically more resistant to critical antibiotic groups: quinolones, drugs like ciprofloxacin; 3rd generation cephalosporins, drugs like cefotaxime and ceftazidime, drugs that have been standard treatments for severe infections, for example kidney infections (pyelonephritis) or bacteraemia' [14]. This has not just been a British phenomenon, but has occurred Europe-wide, and in many non-European countries. The rise in the most recent years has been described by the European Centre for Disease Prevention and Control (ECDC) as 'remarkable' [29].

Graph 3 Increasing rates of resistance (%) to key antibiotics in *E. coli* blood-poisoning infections in England, Wales and Northern Ireland (sources: [15][16][17][27][28])



The average figures hide significant variations between hospitals. Data for individual hospitals is available for Wales [30] and shows that in some hospitals resistance in *E. coli* blood poisoning was as high as 23.6% for 3rd generation cephalosporins, 38.5% for fluoroquinolones and 14.3% for gentamicin.

It is worth noting that the large increases in resistance to fluoroquinolones and modern cephalosporins in human *E. coli* occurred after these antibiotics were licensed for use in farm animals in the mid-1980s and early 1990s: fluoroquinolones were licensed for use in farm animals in the 1991, whereas one modern cephalosporin was licensed in 1985 and the others in the 1990s [31], and the first (and main) modern cephalosporin available for use in pigs was licensed in 1993 [32].

In addition to being the most common cause of blood poisoning, *E. coli* are the main cause of urinary-tract infections in both hospitals and in the community, causing approximately 70-80% of such infections [14]. In a study carried out in London it was found that levels of resistance in *E. coli* causing urinary-tract infections were 'extremely high' for all but one antibiotic used for empirical treatment⁴. Levels of resistance to two antibiotics, ampicillin and trimethoprim, were sufficiently high for the scientists to consider them unsuitable for continued empirical use [33].

While it is generally accepted that antibiotic resistance has been increasing for many years in urinary-tract infections acquired in the community, as well as those acquired in hospitals, there are few reliable data that can be used to establish national trends over time. The Health Protection Agency was unable to provide us with this information. In part this may be because urine samples are not routinely sent for sensitivity testing when uncomplicated urinary-tract infections are suspected by GPs. There is a 10-fold variation amongst GPs in the use of microbiology laboratories in England in relation to acute

⁴ Treatment based on observation and a best guess, which is begun before laboratory tests reveal whether the bacteria are sensitive to the antibiotic chosen

uncomplicated urinary-tract infections [34] . And, a study by HPA and other scientists in 2006 recommended that money could be saved by not routinely testing urine samples in these cases, because the cost of testing was greater than the cost of the prescribing trimethoprim, a first-choice antibiotic [35].

However, a report from the Antimicrobial Resistance Programme Surveillance Unit of Public Health Wales provides data on resistance levels in community-acquired urinary-tract infections caused by coliforms between 2005 and 2010 [30]. Since 70-80% of these are likely to have been caused by *E. coli* [14], this does at least provide some indication of the trend that is likely to have occurred in *E. coli*. The data show that, during the five-year period, resistance to co-amoxiclav increased from 9.4% to 14.6%, for trimethoprim from 26.8% to 31.5%, for fluoroquinolones [such as ciprofloxacin] from 5.8% to 9.7% and for 1st generation cephalosporins from 7.1% to 10.1%. However, here too there are large regional variations, with resistance levels in some areas very much higher than elsewhere. In the area served by the Royal Glamorgan Hospital, for example, resistance to co-amoxiclav and 1st generation cephalosporins were 37.8% and 23.8% respectively, considerably more than double the Welsh average, and resistance to all other tested antibiotics was also significantly higher than the Welsh averages [30].

Resistance to 3rd generation cephalosporins is not usually monitored in community-acquired *E. coli* infections because GPs do not usually prescribe these antibiotics [36]. However, a study in London found that in 2005-2006, 5.7% of community-acquired *E. coli* urinary-tract infections were resistant to the 3rd generation antibiotic cefpodoxime, compared with 21.6% when they were acquired in a hospital [33].

Trying to establish the source of increasing levels of resistance to all types of antibiotics is complicated by several factors. First, it is recognized that in a proportion of cases the patient will have been in hospital during the previous six months, and it has been found that subjects with trimethoprim-resistant urinary-tract infections were 64% more likely to have been in hospital during the previous 180 days [37]. Prior use of the same, and sometimes other antibiotics, also increases the risk of having a trimethoprim-resistant *E. coli* urinary-tract infection, as does having a family member with an antibiotic-resistant infection [37].

However, in a study looking at trimethoprim-resistant *E. coli* UTIs in 2001, 35% of patients with such infections had not been in hospital during the previous 180 days and had not previously been treated with trimethoprim or other antibiotics. While the study's authors acknowledge that other factors can also increase the risk of an antibiotic-resistant infection, including foreign travel they say that, 'The most likely source of drug-resistant bacteria is food, which may contain strains of *E. coli* that are multiply drug resistant' [37]. They see this as the main way in which travellers acquire antibiotic-resistant strains of *E. coli* while abroad.

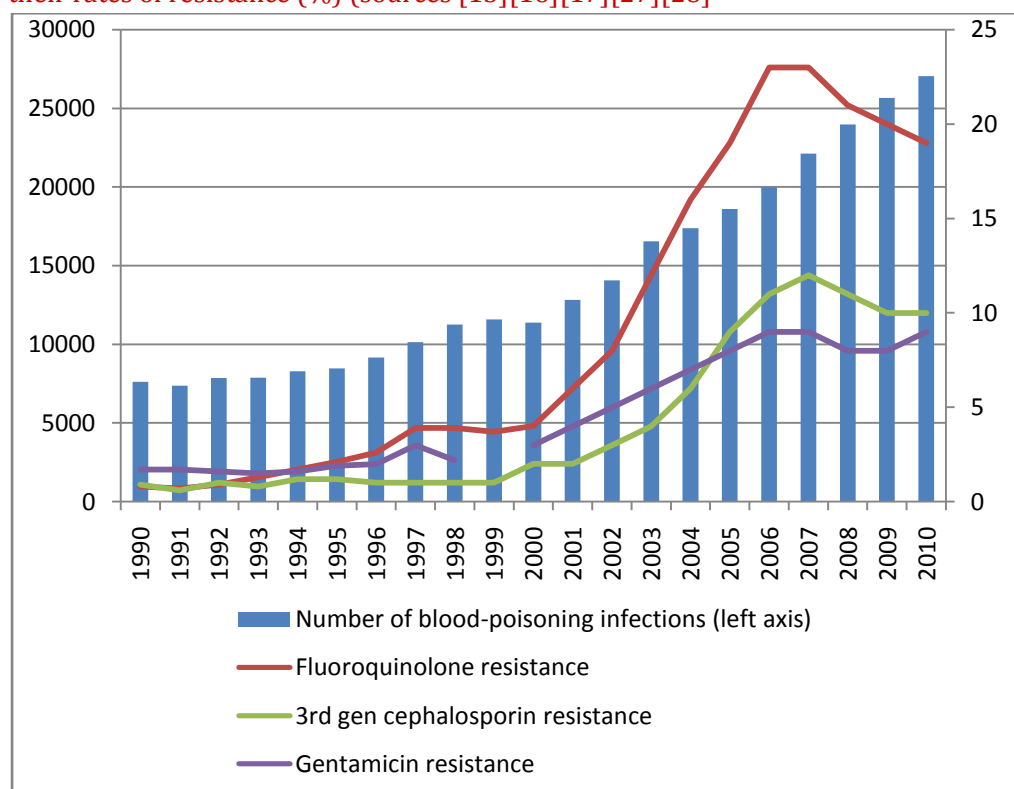
Since 3rd generation cephalosporins are not usually prescribed by GPs to treat community-acquired UTIs, the finding that 5.7% of cases were resistant to cefpodoxime suggests that this resistance has either come from prior hospital usage or from food, or a combination of both.

In this context it may perhaps be significant that in Welsh primary care, resistance has increased in coliforms (predominantly *E. coli*) to all antibiotics since 2005, except nitrofurantoin. Nitrofurantoin is a nitrofuran and is recommended as a first-line treatment for urinary-tract infections, and at a local health board level in Wales, more nitrofurantoin is used than the total of 2nd and 3rd generation cephalosporins and aminoglycosides together. However, while all the antibiotics to which resistance has increased are also used in food-animal production, all nitrofurans have been prohibited from use in food production animals since 1995. It is not clear from the data whether nitrofurantoin use has been increasing in Wales

(in Scotland use increased by 60% in 2010 [19]), but levels of resistance in coliform urinary-tract infections in Wales fell slightly from 11.2% in 2005 to 10.3% in 2010 [30].

High levels of resistance in *E. coli* causing urinary-tract infections can have a major impact on human health, by increasing the risk of treatment failure, the development of a blood-poisoning infection and the rate of hospital admission [11]. This probably explains why the accelerating increase in the number of blood-poisoning infections from 2001 onwards has coincided with the sharply increasing rates of resistance. See Graph 4.

Graph 4 Number of *E. coli* blood-poisoning infections in England, Wales and Northern Ireland and their rates of resistance (%) (sources [15][16][17][27][28])



After rising rapidly during most of the last decade, the average level of resistance to modern cephalosporins, in *E. coli* blood-poisoning infections in the UK, which is mainly accounted for by ESBL resistance, has fallen slightly from about 12% in 2007 to 10% in 2010. Levels of fluoroquinolone resistance have also fallen slightly from their peak levels in 2006-7, see Graphs 3 and 4.

This is believed to be associated with lower use of modern cephalosporins and fluoroquinolones in human medicine over the same period [38][39], probably as a result of the introduction, in 2006, of legislation requiring NHS bodies to adopt antibiotic-prescribing policies aimed at reducing the incidence of hospital infections such as MRSA, *Clostridium difficile* [40][41]. Less encouragingly, the fall in the use of these antibiotics may also be partly due to the rising resistance rates, and the decision by doctors, guided by national prescribing policy, increasingly to use the main antibiotics of last-resort, the carbapenems for empirical treatment.

The reason ESBL and other types of antibiotic resistance in *E. coli* cause such treatment problems, is explained by David Livermore of the Health Protection Agency. He says 'It takes two to three days to culture the blood and find out what it is sensitive to. So treatment is started blind, started empirically'.

He also says that, 'There are numerous studies now to show that if you are given an ineffective antibiotic for a bacteraemia [serious blood-poisoning infection] caused by one of these multi-resistant gram-negatives [such as *E. coli*] you are twice as likely to die as if you are given an effective antibiotic'. He goes on to explain that this creates a pressure to use the last few effective antibiotics which in turn is increasing the rate at which resistance develops to these too [14].

This explains how ESBL resistance in blood-poisoning and urinary-tract infections has led to increasing use of the carbapenems, and since 2008 the HPA reference laboratory has been receiving small but increasing numbers of *E. coli* and *Klebsiella*⁵ resistant to carbapenems [42]. Although many of these carbapenem-resistant bacteria are currently associated with foreign travel, cases with no overseas links are now emerging in *Klebsiella* and other related bacteria [43].

As a result, the lack of any new antibiotics coming onto the market for treating such infections is now a major cause of concern [14].

⁵ *Klebsiella* are bacteria can cause infections such as pneumonia, urinary-tract infections and surgical-wound infections. They are members of the *enterobacteriaceae* family of bacteria, which also includes *E. coli* and *Salmonella*.

4. Antibiotic resistance in human *E. coli* infections can be of farm-animal origin

Antibiotic treatment is frequently recommended for *E. coli* urinary-tract infections, and always used for blood-poisoning infections, so the acquisition of antibiotic resistance can have a significant clinical impact.

However, these *E. coli* are not necessarily acquired through food: urinary-tract infections are generally caused by *E. coli* which are normal, and usually non-disease causing, inhabitants of the human gut. Some strains of these *E. coli*, the extra-intestinal pathogenic *E. coli* (ExPEC) may cause disease if they enter parts of the body which are usually sterile, such as the urinary tract, or the blood stream.

This makes it difficult to trace the origin of the *E. coli* causing the infection. When bacteria like *Salmonella*, or the food-poisoning *E. coli* O157, which are intrinsically pathogenic, establish themselves in the human gut they quickly cause disease. If several people in the vicinity experience the same symptoms, and it can be shown they have eaten the same food, tracing both the source of the disease and potentially also its antibiotic resistance, is straightforward.

For urinary-tract infections, on the other hand, the bacteria might have been a normal, human-adapted *E. coli*, which acquired its resistance when the person took an earlier course of antibiotics; it might have been contracted directly from a close contact or even a family pet; or it might have been a resistant animal *E. coli* acquired through food consumption. A further possibility is that it might have been a human *E. coli* which had acquired a resistance plasmid, a small loop of DNA, which is separate from the bacterium's chromosome and which can carry antibiotic-resistance genes, from an ingested animal *E. coli*. Plasmids can reproduce inside bacteria and copies can then be transferred to other bacteria, thus spreading the genes which give rise to the antibiotic resistance. In this case, although the infection would not be due to an animal *E. coli*, the resistance of the bacteria would be due to farm-animal use of antibiotics.

These complications explain why it has been difficult to establish the extent to which the farm use of antibiotics contributes to resistance in these types of *E. coli* infections. Certain scientists, including scientists funded by the pharmaceutical industry, have used the fact that some studies have found differences in the strains of *E. coli* which colonise the gut of farm animals and of humans, to argue that resistance in farm-animal *E. coli* is largely irrelevant to human health [44].

However, this view is now disputed by many scientists as evidence mounts that a very significant proportion of the resistance in *E. coli* causing urinary-tract and blood-poisoning infections in humans is of farm-animal origin [45][46][47]. The studies presented in the rest of this chapter, taken together, show that there is now compelling evidence that food animals are a reservoir for both antibiotic-resistant pathogenic and commensal *E. coli*, colonising or infecting humans, and also a reservoir for resistance genes which can transfer to *E. coli* which can cause infections in humans. This accumulating evidence has led one leading Australian scientist to warn that, with resistant *E. coli*, 'We are what we eat' [45].

4.1. Evidence from studies with antibiotics used only in farming

Some of the strongest evidence that resistance in human *E. coli* can originate in farm animals occurs when certain antibiotics are used in veterinary medicine but not in human medicine. One antibiotic, a streptothricin called nourseothricin, was used in pigs in the former East Germany in the 1980s, but no

equivalent antibiotics were used in humans over the same period. Resistance to the antibiotic was first detected in porcine *E. coli*. The resistance gene was carried on a plasmid and later resistance was found in *E. coli* from pig farmers. In subsequent years, resistance was found in *E. coli* and other pathogens, such as *Salmonella* and *Shigella*, from people in the wider community [48][49]. One scientist from the UK's Veterinary Laboratories Agency (VLA) commented that 'These observations strongly support the premise that resistance genes present in the commensal flora of animals can spread to bacteria which can colonize or infect humans' [49].

A further example comes from the use of another aminoglycoside, apramycin, which was licensed in the UK for animal use only in 1980. *E. coli* with high-level resistance to apramycin have an apramycin resistance plasmid which is transferable between bacteria. This gene enables the *E. coli* to produce an enzyme called AAC(3)IV, which allows them to resist the action of the antibiotic. Prior to the introduction of apramycin to farming in the UK, no cases of highly apramycin-resistant *E. coli* were found in humans (although cases with lower-level resistance were found). The first known British human case that was highly resistant occurred in 1983, and subsequently they have been found increasingly frequently [50][51]. The public-health significance of apramycin resistance in *E. coli* is that it makes the bacteria also resistant to certain other aminoglycosides, such as gentamicin, which is an important antibiotic used for treating certain *E. coli* infections, such as *E. coli* infections in newborn babies or complicated urinary-tract infections.

In 1994, scientists working for the then Central Public Health Laboratory (now part of the HPA) found that 27% of gentamicin-resistant *E. coli* from humans were apramycin-resistant, produced the AAC(3)IV enzyme, and their apramycin resistance gene was on a transferable plasmid. They concluded that their findings 'support the view that resistance to gentamicin and apramycin in clinical isolates of *E. coli* results from the spread of resistant organisms from animals to man, with subsequent inter-strain or inter-species spread, or both, of resistance genes on transferable plasmids' [52]. In 1986, French scientists had found similar results after apramycin was introduced into French farming in the early 1980s [53].

After reviewing the evidence for the spread of resistance to nourseothricin and apramycin in *E. coli*, government scientists from Denmark and a scientist from Australia commented in 2008 that 'these observations strongly indicate that resistance to streptothricin and apramycin emerged primarily among food animals because of the selection by the use of these antibiotics for food animals and that, subsequently, resistant bacteria were transmitted to humans' [3].

4.2. Animal *E. coli* can colonise the human gut and transfer resistance genes to human *E. coli*

An early study published in 1969 involving attempts to colonise the human gut by giving a volunteer large oral doses of animal *E. coli* had concluded that animal *E. coli* were poor colonisers of the human gut [13]. However, other scientists pointed out that this experiment was limited to just one human volunteer and a small number of animal strains [54].

Subsequent British research, published in 1977, provided conclusive evidence that *E. coli* of farm-animal origin could establish themselves in the human gut after the handling and eating of food [54]. Four volunteers prepared, cooked and ate chicken bought from local retailers. The *E. coli* gut bacteria of the volunteers were monitored for several weeks. Each participant handled an average of three birds during this period. The *E. coli* contamination of the birds was also monitored. The *E. coli* from the volunteers and the birds were compared for antibiotic-resistance profile, strains (by a method known as serotyping), and the plasmid DNA was also compared. One of volunteers was found to have been colonised by five *E. coli*

strains of poultry origin. Four of the poultry strains were isolated the day after the volunteer had handled and prepared the meat, but before any meat had been eaten. The *E. coli* could not be found in the volunteer's faeces before handling the meat. Analysis of the plasmids in the *E. coli* showed that these were identical in the volunteer and the bird. The strains all persisted in the volunteer's gut for five days, and one strain for ten days.

This evidence was considered sufficient to establish beyond reasonable doubt that the avian *E. coli* had established itself in the volunteer's gut flora. It was also concluded that merely handling contaminated meat could be sufficient to result in gut colonization [54]. This finding suggests that, although cooking meat for long enough at high enough temperatures will kill *E. coli*, humans could still be exposed to resistant animal *E. coli* by handling meat.

More recent Danish research published in 2009 showed that antibiotic-resistant pig strains of *E. coli* ingested by human volunteers were able to colonise the gut of eight of the nine volunteers for two weeks without any antibiotics being administered [55].

Furthermore, scientists have found the same resistance plasmids have been detected in animal and human *E. coli*, which suggests that transfer of these plasmids from one strain to another has occurred [46]. Several studies, using animal models, have shown that transfer of resistance genes, including from animal to human *E. coli*, can occur in the intestine [56][57]. Some studies have also shown that ingested animal *E. coli* can transfer resistance plasmids to resident human *E. coli* in the human gut [13][58]. A Danish study carried out on a pig farm, and published in 2009, found that *E. coli* from pigs, pig farmers and the environment were genetically varied, but carried indistinguishable or very closely related ESBL CTX-M plasmids. They concluded that the plasmids were being transmitted from the pig *E. coli* to various strains of human *E. coli* [59].

Danish government scientists believe that taken together, these studies show that 'the transfer of resistance genes between *E. coli* of animal and human origin in the intestine of humans is very likely' [46]. Research has also shown that gene transfer between animal and human *E. coli* could be occurring in the kitchen, even before any meat is consumed. In various experiments, Norwegian scientists found that *E. coli* from cattle transferred a resistance plasmid to human *E. coli* on a towel, pig *E. coli* transferred resistance to human *E. coli* on minced meat, and bacteria from fish were able to transfer resistance to human *E. coli* on a chopping board [60].

Several studies have also shown that the administration of antibiotics to the humans or animals ingesting resistant *E. coli* could better enable these *E. coli* to establish themselves in the gut, or could lead to increased rates of gene transfer to the resident *E. coli* [56][57][58][61]. This suggests that in some cases, while the original resistance will be of animal origin and associated with farm-animal use of antibiotics, the subsequent human use of antibiotics can help the resistant animal *E. coli* establish itself in the human gut, or help increase the transfer of resistance genes to human *E. coli*. In such cases, both the human and animal uses of antibiotics will be associated with the dissemination of resistant *E. coli* in humans.

An American study compared mobile genetic elements called integrons, and their antibiotic-resistance gene cassettes⁶, from human *E. coli* which had caused urinary-tract infections and from farm-animal *E. coli*. In 35 of 65 (54%) human *E. coli* with antibiotic-resistance gene cassettes, the DNA sequences were 100% identical to the corresponding cassettes in *E. coli* from food animals. The authors said that:

⁶ A gene cassette is a DNA sequence containing one or more genes which code for a single type of function, such as antibiotic resistance. Integrons are genetic structures in bacteria which are capable of acquiring and exchanging gene cassettes.

'These data suggest that food-producing animals are a major reservoir for integrons carrying antimicrobial drug-resistant genes. They may also serve as a source for the transfer of these genes not only to *E. coli* and *Salmonella* but also to other members of Enterobacteriaceae and other bacterial species. Thus, it is not only the spread of drug-resistant strains but also the spread of mobile elements carrying drug resistance genes across bacterial species that contributes to the changing prevalence of drug-resistant human infections in different regions of the world. That is, there appears to be a global "epidemic" of mobile drug resistance genes, possibly spread by globalization of food trade. This phenomenon could affect not only community-acquired infectious diseases, such as UTI [urinary-tract infections] and gastroenteritis, but also health care-associated infections' [62].

4.3. *E. coli* and food – poultry an important source of resistant *E. coli*

A French study, published in 1988, involved feeding six volunteers a near-sterile diet for an average of 17 days, after an earlier control period of 21 days. During the control period, they were fed their usual diet, and for the sterile-diet period their food was heated to 105 degrees centigrade for one hour, which was shown to be sufficient to destroy *E. coli* bacteria. The day after the sterile diet began, the number of *E. coli* in the volunteers which were resistant to ampicillin, streptomycin, and tetracycline fell, and it reached a minimum in just three days. The fall in the number of resistant *E. coli* was much larger than the slight fall in the number of sensitive *E. coli*, which was not statistically significant [63].

These French findings are consistent with more recent research carried out in Spain and the United States. Scientists in Spain collected blood and faecal *E. coli* samples from people in Barcelona and compared them to faecal *E. coli* taken from geographically matched chicken [64]. They tested the *E. coli* for resistance to fluoroquinolones, important antibiotics which are used for the treatment of *E. coli* and other infections. They then analysed the genetic make-up of all the *E. coli*. They found that resistant human isolates were distinct from the sensitive human isolates, but that they were largely indistinguishable from the poultry isolates. In contrast, the resistant and sensitive poultry *E. coli* were genetically similar. They said that the results were consistent with the hypothesis that fluoroquinolone-resistant *E. coli* were arising in poultry as a result of antibiotic use in poultry and that 'many of the fluoroquinolone-resistant *E. coli* encountered in humans may be imported from chickens, rather than having originated in humans'. They said that their study, published in 2006, provided the 'strongest molecular evidence available to date for a food (specifically chicken) source for potentially pathogenic fluoroquinolone-resistant *E. coli* in humans' [64].

An Australian scientific adviser to the WHO and a scientist working for the US Center for Disease Control and Prevention commented that this Spanish study provided further strong evidence that resistant *E. coli* of animal origin could colonise the human gut [65]. They also said the fact that fluoroquinolone resistance in human *E. coli* in Australia was at much lower levels (3.4%) [66] than it is in the United States (18.9%) [67] may provide further evidence that food animals are an important source of fluoroquinolone resistance in human *E. coli*. Fluoroquinolones have never been licensed for use in Australian farm animals, whereas they are used in food animals in the US (although the US banned the use of fluoroquinolones in poultry in 2005), but fluoroquinolones have been used in humans and pets in Australia for over 20 years [45][65]. In the UK, where fluoroquinolones are used in farm animals, approximately 20% of *E. coli* from human blood-poisoning infections over the past few years have been resistant to fluoroquinolones (see Graph 3), a rate six times higher than in Australia.

A very similar study carried out in the United States, and published in 2007, provided findings consistent with the Spanish study. *E. coli* from infected patients and from healthy vegetarians were compared with *E. coli* from poultry meat. Isolates resistant to the fluoroquinolones, modern cephalosporins (two classes

of antibiotics classified as critically important in human medicine by the WHO and associated with the spread of ESBL *E. coli*) and the antibiotic combination trimethoprim-sulfamethoxazole were compared with susceptible *E. coli*. The scientists again found that the resistant human *E. coli* were genetically unrelated to the sensitive human bacteria, whereas they were similar to bacteria from poultry. On the other hand the resistant and sensitive *E. coli* from poultry were largely indistinguishable. They concluded that 'Many drug-resistant human fecal *E. coli* isolates may originate from poultry, whereas drug-resistant poultry-source *E. coli* isolates likely originate from susceptible poultry-source precursors' [68]. They said their results were consistent with the earlier Spanish research, but extended the finding to classes of antibiotics other than the fluoroquinolones. They also, perhaps surprisingly, found that the similarity with poultry *E. coli* was also true for the isolates taken from vegetarians. This may have been due to other members of their household preparing and eating poultry products, and the *E. coli* then spreading from the household environment to the vegetarians.

A second US study carried out in rural Idaho compared the phylogenetic groups and virulence genes of *E. coli* from 217 faecal samples from humans with that of *E. coli* from 231 meat samples (beef and poultry). Isolates with the same phylogenetic group and virulence profile were then analysed by PFGE and random amplified polymorphic DNA (RAPD) analysis. As in the two earlier studies they found that the resistant stool samples were more similar to the resistant meat samples than the sensitive stool samples. They said 'This is consistent with the resistant stool isolate population possibly having originated in meats, rather than by conversion of susceptible human associated strains to resistance'. They also found that nearly 20% of the meat isolates were ExPEC and concluded that 'The observed molecular similarity of certain meat and human-source *E. coli* isolates, including antimicrobial-resistant and potentially pathogenic strains, supports possible foodborne transmission' [69].

Icelandic research, published in 2010, also compared fluoroquinolone-resistant *E. coli* from humans (blood and urine samples), to *E. coli* from live poultry, poultry meat and poultry feed. Using a typing method called pulsed-field gel electrophoresis (PFGE), considered a gold-standard method in epidemiology, they found that the human resistant isolates clustered with the poultry, poultry-meat and poultry-feed isolates. They said that this implicated poultry and poultry meat as a source of human fluoroquinolone-resistant *E. coli*, a finding which, they said, was consistent with earlier studies [70].

4.4. Farmers at increased risk

A Dutch study published in 2001, like the earlier studies with nourseothricin and apramycin, found evidence that resistant *E. coli* could spread from farm animals to farmers, and at a slower rate to the wider community. The scientists tested broiler chickens, turkeys, laying hens, broiler farmers, turkey farmers, laying hen farmers, broiler slaughterers and turkey slaughterers. Laying hens receive far fewer antibiotics than broilers and turkeys, so unsurprisingly they found lower levels of resistance in *E. coli* from the laying birds than from the other birds. Significantly, they also found that resistance to nearly all of the antibiotics was lower in the *E. coli* from the laying-hen farmers than from the other farmers and the slaughterers. Multiresistance was common in broiler and turkey farmers, but absent from laying-hen farmers. The same resistance patterns were found in broilers, broiler farmers and broiler slaughterers and in the turkeys, turkey farmers and turkey slaughterers. They also found the same *E. coli* on turkey meat as they had isolated from turkey. The scientists concluded that 'The results in this study strongly suggest a spread of antibiotic-resistant *E. coli* from animals to people – not only to farmers but also at a lower level to the consumers of poultry meats' [71].

More recently, a 2007 study in the United States showed that poultry workers are 32 times more likely to carry gentamicin-resistant *E. coli* than other members of the community. They also had a significantly increased risk of carrying multidrug-resistant *E. coli* [72].

4.5. Human extra-intestinal pathogenic *E. coli* and farm-animal *E. coli*

Research published in the past few years has also strengthened the evidence that extra-intestinal pathogenic *E. coli* involved in urinary-tract infections, particularly resistant *E. coli*, are frequently of farm-animal sources. That this might be the case has been strongly suspected for a long time due to outbreaks of urinary-tract infections caused by a single *E. coli* strain, with unrelated people being affected, sometimes over a large geographical area. Such outbreaks have occurred in the UK, Denmark, Spain the United States [62][73][74] [75].

E. coli are the most common bacterial pathogen in poultry, and extraintestinal *E. coli* infections in poultry, such as colibacillosis, are a frequent occurrence in intensively farmed birds. These are caused by *E. coli* strains referred to collectively as ‘avian pathogenic *E. coli*’ (APEC). Studies have compared APEC with *E. coli* causing urinary-tract infections in humans using a typing method known as multilocus sequence typing (MLST). One American study found that at least some of them are highly similar, and the scientists concluded that their data ‘supports the possibility that a food-borne link between some APEC and UPEC strains exists’ [76]. A German study found that faecal *E. coli* from chickens, which were most virulent in chicken-infection experiments, belonged to MLST sequence types that were almost exclusively associated with extraintestinal diseases not only in birds but also in humans (this included diseases like septicemia, urinary-tract infection, and newborn meningitis). They concluded that some faecal *E. coli* strains from chickens can infect humans [77].

Apparent confirmation of some of these findings came in another American study which compared *E. coli* from women with urinary-tract infections to *E. coli* from retail meat using four different typing methods. Two the *E. coli* strains isolated from retail chicken were found to be indistinguishable or closely related to human *E. coli* causing urinary-tract infections. The scientists said their study provided ‘strong support for the role of food reservoirs or foodborne transmission in the dissemination of *E. coli* causing common community-acquired UTIs [urinary-tract infections]’. They also said that it is ‘probable that a food reservoir exists and that foodborne transmission of extraintestinal *E. coli* is common’ [78]. However, other scientists reviewing this last study argued that the evidence did not strongly support the hypothesis that retail chicken was the main reservoir for human extra-intestinal *E. coli* infections, although they said the results were ‘noteworthy’ and they highlighted the importance of further research on the issue [79].

A Spanish study, compared *E. coli* isolates of serotype⁷ O1:K1:H7/NM from humans and from poultry from several countries. The ExPEC human strains had caused either meningitis in newborn babies, urinary-tract infections or blood poisoning, and the APEC poultry strains had caused colibacillosis in the birds. For many of the isolates, they found differences in the phylogenetic groups and virulence genes. However, they found one subclone containing both human and animal strains from two countries which had the same MLST type, the same phylogenetic group, the same virulence genes and the same PFGE cluster. They concluded that *E. coli* from poultry ‘may act as potential pathogens for humans’, and that these pathogenic bacteria may be transmitted to humans via food [80].

American researchers also compared APEC strains from poultry with *E. coli* which had caused meningitis in newborn babies. They found that ‘they were not easily differentiated on the basis of multilocus

⁷ Bacteria are classified into serotypes according to what ‘antigens’ they have on their cell surface. An antigen is a substance which causes our immune systems to produce antibodies to combat the bacteria.

sequence typing, phylogenetic typing, or carriage of large virulence plasmids', and that using a rat model of human neonatal meningitis, some of the APEC strains were able to cause meningitis. They concluded that some findings supported the hypothesis that some, but not all APEC strains had 'zoonotic potential', ie. they can be passed from animal to human and cause disease in humans [81].

A series of recent scientific papers by Danish government scientists has provided 'solid evidence that urinary-tract infection is at times a zoonosis' [82]. *E. coli* from humans and animals can be divided into 'phylogroups' A, B1, B2, D and non-typeable. Most *E. coli* urinary-tract infections are caused by phylogroup B2, and less frequently urinary-tract infection can be caused by phylogroup D, A and B1 in that order. Analysis of *E. coli* from pigs, broiler chickens, pork and chicken meat found that phylogroup A was the most common, but all phylogroups, including B2 and D were found. The animal *E. coli* were analysed for virulence genes known to be associated with extraintestinal infections in humans, and seven of eight virulence genes searched for were found in pigs, pork, broilers and chicken meat. All of 13 *E. coli* isolates in phylogroup B2 taken from the farm animals or meat were then shown to be able to cause urinary-tract infections in mice, as were 15 of 25 animal or meat *E. coli* from phylogroup D. Since these mice models are considered to be representative of human UTI, the authors said that this was 'important circumstantial evidence that UTI [urinary-tract infection] is a zoonosis' [83][84][85][86].

A scientific review covering many of these studies and the studies on retail meat, but not all of the most recent Danish studies, found that 'These findings reinforce the hypothesis that some human ExPEC diseases could arise from poultry and pig ExPEC reservoirs following contamination of the meat in slaughterhouses or in retail-food markets.' They also warned that 'ExPEC could be transmitted from poultry to humans through meat consumption, but another plausible route of transmission from animal reservoirs to humans is via environmental contamination from manure or faeces of wild or domestic birds'. The authors of the review were particularly concerned about the levels of antibiotic resistance in animal *E. coli* being transmitted from animals to humans saying 'In recent years, there have been accumulating data that support the likelihood that animal reservoirs could be responsible for contamination of humans with antimicrobial-resistant ExPEC and other bacteria through the consumption of contaminated food'. They called for measures to reduce ExPEC in animals and the use of antibiotics in farm animals and people [87].

4.6. Strong correlation between resistance rates in human and animal *E. coli*

Research published in December 2011 by scientists from Australia, Canada and Denmark analysed the association between resistance to four antibiotics, or families of antibiotics, in *E. coli* from pigs, poultry and cattle and *E. coli* from blood-poisoning infections in humans. They also compared rates of resistance in the human bacteria, with antibiotic usage in humans in the different countries. The antibiotics examined were ampicillin, aminoglycosides, 3rd generation cephalosporins and fluoroquinolones.

In all four cases, they found strong and statistically significant correlations between resistance rates in *E. coli* from poultry and resistance rates in *E. coli* from humans, as well as similar strong and statistically significant correlations for ampicillin, aminoglycosides and fluoroquinolones for the pig and human *E. coli*. For cattle, only resistance to ampicillin was statistically significantly correlated with resistance in humans [47].

Human antibiotic use was only significantly correlated to human resistance rates for the fluoroquinolones and the 3rd generation cephalosporins. The authors said that the relationships found between resistance in animal and human *E. coli* may be an indication of the transference of resistant bacteria via the food

chain, but could also be a consequence of similar within country use of antibiotics in humans and animals. However, for countries where the data was available, they did not find 'any clear correlation between animal and human antimicrobial usage patterns, which supports the hypothesis of foodborne transference of resistant bacteria'. They concluded 'these findings exclude [human] antimicrobial usage as the only explanatory variable for the observed resistances in *E. coli* from humans. They suggest that, in addition to the contribution of antimicrobial usage in people, a large proportion of resistant *E. coli* isolates causing blood-stream infections in people are likely to be derived from food animal sources' [47].

5. ESBL *E. coli* in hospitals and the community

In 2003, diagnostic laboratories throughout Britain began advising the Health Protection Agency (HPA) that they were encountering numerous cases of ESBL *E. coli* infections from both hospital and community patients [88][89]. Frequently the cases were urinary-tract infections, but sometimes blood-poisoning infections occurred [90].

ESBL infections can be difficult to treat because the resistant *E. coli* produce enzymes which destroy large number of antibiotics, including penicillins and cephalosporins, two of the most important classes of antibiotics available to medicine. Very few antibiotics are still effective against the bacteria, and for serious infections, the antibiotics may need to be given by injection.

Although ESBL *E. coli* infections had occurred before 2003, from 2003 onwards a new type of ESBL, called CTX-M ESBL, became predominant. While all CTX-M plasmids carry genes making them resistant to penicillins and cephalosporins, many also carry a wide range of other resistance genes making them additionally resistant to other extremely important classes of antibiotics, such as the fluoroquinolones and aminoglycosides. As a result, according to the HPA, CTX-M *E. coli* are 'exceptionally resistant to multiple antibiotics', and their resistance includes an even wider range of antibiotics than for other ESBLs [26]. The CTX-M resistance genes are carried on various plasmids, many of which can be transferred to other bacteria, thus spreading the genes which give rise to the antibiotic resistance.

According to Defra and HPA scientific advisors, CTX-M ESBL *E. coli* are more virulent [10]. Furthermore, as a result of the high levels of resistance to antibiotics, and because of delayed identification of the cause of the infection by some laboratories, death rates can be high [90]. In 2006, the Chief Medical Officer reported that a study of community-acquired ESBL *E. coli* urinary-tract infections had found a fatality rate of approximately 30% [91]. A large outbreak in Southampton in 2003-4 led to 29 deaths, although this was just 8% of cases [90]. A study in a hospital in Salford found that 48% of patients with an ESBL *E. coli* blood-poisoning infection died [92]. A recent study covering hospitals in 13 European countries, including England and Scotland, found that patients with bloodstream *E. coli* infections were 2.5 times more likely to die within 30 days of an infection and 2.9 times more likely to die during their entire hospital stay if the *E. coli* were resistant to 3rd generation cephalosporins (such resistance is mainly due to CTX-M ESBL resistance) [23]. At the same time, resistance to 3rd generation cephalosporins prolonged the average hospital stay by five days.

Earlier types of ESBL *E. coli* which predominated before CTX-M ESBL *E. coli* were almost exclusively restricted to hospitals [11], but CTX-M ESBL *E. coli* mainly affect community patients [10]. Although hospital outbreaks do occur, ESBL *E. coli* is not now considered to be a hospital superbug like MRSA because so many infections are community-associated [93]. The recent cases in a hospital in Swansea, for example, when two babies died as a result of ESBL *E. coli* infections, involved both infections being acquired in the community and subsequent hospital transmission of this community-acquired strain [94].

Modern cephalosporins, the antibiotics which most strongly promote the spread of ESBL *E. coli*, are usually only prescribed by hospital doctors treating serious cases of infection, and not normally by GPs seeing patients in their surgeries. The community origin of many ESBL infections therefore raises the question of whether farm animals and the food chain could be a possible source of the bacteria. In 2005, the HPA published a report looking at the increasing problems being caused by the emergence of ESBL *E. coli* [26]. It suggested that because studies had shown that ESBL *E. coli* was sometimes present in human

faecal matter, this 'may imply spread via the food chain'. Dr Georgina Duckworth, an author of the report, said: 'The findings in our report show evidence of people carrying these bacteria in their gut. If this is found to be commonplace in the general population this may point towards the food chain being a potential source' [95].

At the time of the publication of the HPA report, one study had found that 1.4% (8 of 565) of community-based patients had ESBL *E. coli* bacteria in their faeces, whereas just 0.25% (1 of 394) of hospital-based patients had the bacteria⁸ [96]. Another study published in 2004, however, found that 76% of patients who acquired an ESBL *E. coli* infection were hospital patients or had recently had contact with a hospital, and just 24% were classified as community-acquired [88]. This may seem a surprisingly high percentage of hospital infections since *E. coli* are not bacteria that patients acquire from the hospital environment as frequently as with some other bacteria. The authors of a 2007 paper, including scientists from the HPA, suggested that 'it may be that low-level gut colonization occurs in the community [by ESBL *E. coli*], via the food chain, perhaps with plasmid transfer to resident *E. coli*, and that the proportion of resistant *E. coli* with CTX-M enzymes tends to be enriched during healthcare contacts, owing to frequent antimicrobial exposure' [97].

Recent research carried out in Birmingham found that 11.3% of community patients (GP patients or outpatients) had ESBL *E. coli* in their faecal matter [98], a very large increase over earlier findings, and perhaps pointing towards the foodchain as a possible source, as Dr Duckworth had suggested.

The emergence of ESBL *E. coli* in the community is not just a British phenomenon. Canada, Croatia, France, Hong Kong, Israel, Italy, Spain, South Korea and the United States have all reported the emergence of this new superbug in the community [99][100][101][102][103].

While elderly patients are at present those most at risk (see Graph 2), HPA scientists have suggested that this might change over time. They point to a study in Hong Kong which found significant levels of ESBL resistance in *E. coli* urinary-tract infections in women of all ages. They also say that a Canadian study found a much broader age distribution in community-acquired ESBL *E. coli* infections than in hospital acquired ESBL *E. coli* infections. The HPA scientists say that this raises new concerns. They say: 'Rising rates of *E. coli* with CTX-M ESBLs in the genitourinary tracts of sexually active women raise the alarming possibility that these enzymes might "escape" into sexually transmitted bacterial pathogens, specifically *Neisseria gonorrhoeae*. Oral and intramuscular oxyimino-cephalosporins, such as cefixime and ceftriaxone, are widely used as a first-line treatment for uncomplicated gonorrhea, and any evolution of ESBL-producing gonococci would be a catastrophic development' [104].

⁸ Although only one hospital patient carried ESBL *E. coli*, a further 12 carried other bacteria (*Enterobacter cloacae*, *Klebsiella* and *Citrobacter freundii*) with similar ESBL resistance to the *E. coli*. One community-based patient also carried *Salmonella* with ESBL resistance.

6. ESBL *E. coli* in farm animals and on food

The first case of ESBL *E. coli* in British farm animals was found by the Veterinary Laboratories Agency (VLA) in the autumn of 2004, in scouring calves from a dairy farm in Wales. Testing showed that 56% (27 of 48) calves and 3% (2 of 60) cows were positive for the bacteria [105]. Two years later, follow-up testing found calf mortality had increased on the farm and that the percentage of ESBL-positive animals increased to 70% of calves and 57% of cows [106].

The second cattle farm affected was only found in July 2006, as a result of investigations into calf deaths [107]. A genetic analysis of the plasmid, the small piece of DNA carrying the ESBL resistance gene, showed that this was likely to be the same plasmid as carried by the most common strain of ESBL *E. coli* affecting humans, but that the strain of *E. coli* affecting the calves was not the same as this predominant human strain [107][108].

However, ESBL resistance plasmids, like many other resistance plasmids, have been shown to be transferable from one strain of *E. coli* to another [107][109][108][109][110][111]. VLA scientists therefore concluded that the resistance plasmid had transferred between human and cattle *E. coli*, and in the case of this farm, they thought that the direction may have been from human to cattle *E. coli*, rather than the other way round [106][108][112], as this particular plasmid had been found several years earlier in humans [113]. The spreading of the resistance plasmid from human to animal *E. coli* may have occurred because of the presence of human sewage in surface waters or elsewhere [112].

Nevertheless, although this resistance plasmid may not have originated in cattle, it is now widely established and still spreading on British cattle farms. In addition, the fact that this plasmid may have transferred from human to cattle *E. coli* means it is also very likely to be able to transfer the other way as well when, for example, bovine *E. coli* are present on food consumed by humans. Transfer from the cattle *E. coli* to human *E. coli* can then occur in the gut and the resistant human *E. coli* can subsequently cause infections which are not easily treatable [3][97].

After the initial detection of ESBL *E. coli* on the Welsh farm, Defra pointed to the fact that this was the only case then known in British farm animals and said that 'this would suggest that this type of resistance was at a very low level in livestock' [114]. It warned against over-reaction to the findings saying that 'proportionate action should be taken with medical colleagues in the area as the ESBLs found in animals had so far been different from the prevalent types currently found in humans' [115].

In 2005, before several hundred cattle from the original infected farm in Wales were sold at public auction, officials from both Defra and the Welsh Assembly considered the issue, but decided not to impose any restrictions on the sale. Defra stated, 'It is currently unclear what risk ESBL producing bacteria associated with animals pose to public health... Following consultation with public health officials and colleagues from the FSA, it has been decided that additional precautions or on-farm restrictions are not required in this case [107].

However, these apparently reassuring statements were made before any active surveillance for the bug had been carried out. Research carried out by the VLA since then has shown that Defra was wrong in suggesting that ESBL resistance was at a very low level in British farm animals.

A survey of broiler-chicken⁹ abattoirs published in early 2010 found ESBL *E. coli* in chickens at 52% (12 of 23) of abattoirs, and in 3.6% of individual birds. VLA researchers also surveyed boot swabs from turkey farms and found positive samples for 5.2% (16 of 308) of meat farms and 6.9% (2 of 29) of breeder farms [116].

VLA research carried out in 2008/9 and first published at a conference in September 2010 found that 37.5% of randomly selected cattle farms in the North West of England had animals which were positive for ESBL *E. coli*. This finding was described by one of the VLA scientists as ‘completely unexpected’ [117][118]. Furthermore, from a sample of the 160 farms which purchased cattle from the original affected farm in Wales, VLA scientists estimated that 59% of these were affected by ESBL *E. coli* [117], suggesting that the failure of Defra and the Welsh Assembly to impose any restrictions on the sale of cattle from the first affected farm may have contributed the higher incidence of ESBL strains on these farms. Another recently published VLA study screened faecal samples from 113 cattle and/or sheep farms in North Wales and the West Midlands and found six positive cattle samples and two positive sheep samples [118].

Further VLA research, published earlier this year, surveyed seven pig farms, six of which were linked as a network of breeding, growing, and finishing units. ESBL *E. coli* was found on all the farms and in 86.9% (438 of 504) of the faecal samples collected from the animals. The same study compared the numbers of ESBL *E. coli* found in the positive pig samples with those found in the earlier poultry abattoir survey samples and in samples collected on three cattle farms. The scientists found that the median numbers of resistant *E. coli* in the pig and poultry samples were higher than in the cattle samples. They designated animals shedding more than a specific number of ESBL *E. coli* bacteria in their faeces as ‘high-density shedders’. Using their definition, they found that 46.9% (15 of 32) chickens tested, 40% (8 of 20) of pigs and 8.6% (3 of 35) of cattle were high-density shedders. They suggested that these animals may be disproportionately involved in the dissemination of ESBL genes and may pose a greater risk of contamination of carcasses going into the food chain if the animals go to slaughter [119].

6.1. ESBL *E. coli* on food

Despite the serious threat posed by the spread of ESBL *E. coli* on food, there has been no systematic monitoring for ESBL *E. coli* in British food and no testing at all, even for scientific research, since 2006. In January 2007 the Soil Association wrote to Dame Deirdre Hutton, Chair at the time of the Food Standards Agency (FSA) asking her to include ESBL *E. coli* in their food surveys. In reply, she set out the FSA’s view that it was far more likely that ESBL *E. coli* causing human infections were coming from previous hospital admission of other family members rather than food. However, she added, ‘Irrespective of the above information I would like to emphasise that the FSA is concerned about the finding of ESBLs in bacteria from farmed animals and the public health risk this may entail. The Agency is liaising closely with the relevant Government Departments and public health bodies are continuing to monitor the situation. Inclusion of screening for ESBL *E. coli* in forthcoming FSA food surveys is being considered’ [120]. Five years on, the FSA has still not tested food on sale in the UK from the presence of ESBL *E. coli*.

Only one study has ever examined British-produced meat and this covered only chicken. It was carried out in 2006 and involved testing 62 samples of chicken from UK-reared birds. It found that one of the samples was positive for ESBL *E. coli*. The meat was bought from supermarkets in the West Midlands. The same study also found that nine of 27 samples of imported chicken and a further seven of 40 samples of chicken of unknown origin were also positive. The samples imported from Brazil were frequently positive

⁹ Broilers are chickens raised for meat, as opposed to egg-laying.

for a particular ESBL CTX-M plasmid, CTX-M-2, which is the dominant CTX-M plasmid in humans in that country, but currently rare in the UK. However, five of the samples of unknown origin had the CTX-M-14 plasmid, which is the second most common plasmid in humans in the UK [121]. CTX-M-14 has now been found in British poultry [122], so it is possible that some of these positive samples were of UK origin.

A second British study focused exclusively on poultry imported from South America. It found 62 positive samples from 210 tested, and again the dominant resistance plasmid was CTX-M-2 [123].

A study in Northern Ireland examined 54 food samples, most being shellfish and a majority of the rest being cooked meat or cooked restaurant meals. No ESBL bacteria were found, however, the survey did not include any raw meat samples [124].

No British studies at all have tested turkey meat, pork, beef or salad vegetables (except for one salad sample included in the Northern Irish study) for ESBL *E. coli*. While meat is the most obvious food to test, the potential also exists for contamination of vegetables due to the use of inadequately composed manures, or irrigation of crops from rivers or streams contaminated with bacteria from farms or sewage systems upstream. Runoff from farmland into streams following heavy rainfall on land where either animal slurry or human sewage has been spread is a further potential source of contamination [10].

In their recent ESBL report, the government's advisory committees DARC (Defra Antimicrobial Resistance Coordination Group) and ARHAI (Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection) said that sampling of British meat represented 'just one food type from one region therefore this is an area which warrants wider and systematic investigation' [10].

Scientists in some other European countries, however, have already undertaken more extensive testing. Studies abroad have sometimes found a very high proportion of retail meat, particularly poultry, which is positive for ESBL *E. coli*. One Dutch study found that 77% (68 of 89) of chicken meat samples, 5% (5 of 85) of beef samples and 2% (1 of 57) of pork samples were positive for ESBL *E. coli* [125]. A subsequent Dutch study found even higher levels: 94% (92 of 98) of retail chicken meat samples were positive for ESBL *E. coli* [126]. A third, very recent Dutch study has found very high levels of ESBL bacteria on organic retail chicken meat as well as on non-organic chicken. The study found 84% of organic meat samples were positive, compared with 100% of non-organic meat. The number of ESBL bacteria on the positive samples was significantly lower on the organic meat than on the non-organic meat (four times lower) [127]. As we explain in Section 9.3 below, one of the most likely explanations for this high incidence in organic Dutch poultry is that many organic poultry farmers are buying in one-day old chicks which have been hatched from non-organic eggs and already had ESBL *E. coli*. Another possible explanation, also compatible with the lower numbers of ESBL *E. coli* bacteria on organic chickens, is that the organic chickens became contaminated at the abattoir, either before or after they were slaughtered.

A study in Denmark tested a wide range of home-produced and imported meat. Most of the imported chicken in Denmark had come from either Germany or France, and in the case of the German chicken imports, all the chickens had also been raised in Germany. In total, 34% of the German and 43% of the French chickens were positive for ESBL *E. coli*. Only seven of the French chickens were known for certain to have been reared in France, and four of these were positive for ESBL *E. coli* [128]. Danish home-produced chicken had much lower levels of ESBL *E. coli* positives (just 3.3%) [128], probably reflecting the much lower levels of antibiotic use in Danish farming compared with French and German farming [129].

Spanish retail meat has also been shown to have high levels of ESBL *E. coli*: one study found the bacteria in 67% (8 of 12) of chicken samples, 58% (7 of 12) of turkey samples, 25% (3 of 12) pork samples and 8%

(1 of 12) beef samples [130]. An earlier Spanish study had found just three positives out of 738 food samples, however most of these samples were of cooked food, and two of the positives were from salads, with the other being from cooked chicken [100]. A third Spanish study found ESBL producing bacteria in 57% (27 of 47) of chicken meat samples, in 58% (7 of 12) rabbit samples, but found none in 30 pork and 22 veal samples [131].

One of the studies examining Spanish retail meat, also tested retail meat from Pittsburgh in the United States. It found much lower levels of ESBL *E. coli* in the American meat, but higher levels of another type of 3rd generation cephalosporin resistance called AmpC resistance¹⁰: just 5% (1 of 20) of US chicken samples was positive for ESBL *E. coli*, in comparison to 67% of Spanish samples, whereas 85% (17 of 20) of US chicken samples were positive for AmpC resistance compared with none of the Spanish samples. The US survey also found AmpC *E. coli* in 70% (14 of 20) of turkey samples, 10% (2 of 10) of pork samples and 5% (1 of 20) of beef samples, but none of these meats had ESBL *E. coli*. The same study also looked at human *E. coli* infections with resistance to 3rd generation cephalosporins. They found 79 ESBL *E. coli* infections in Seville and just one AmpC *E. coli* infection, whereas they found 25 AmpC *E. coli* infections in Pittsburgh and 22 ESBL *E. coli* infections. The much higher levels of AmpC infections in the US, and much higher levels of ESBL infections in Seville appear to reflect to some extent the kind of resistant bacteria being found in food. The authors said their findings ‘provided an ecological relationship between contamination of retail meat and colonization or infection due to ESBL-producing and CMY-producing *E. coli*, but we could not demonstrate a direct causal relationship’. They concluded that ‘Our findings provide further circumstantial evidence that retail meat may serve as a source of ESBL-producing and CMY-producing *E. coli* isolates, which may then colonize the human intestine and cause infections, or serve as donors of ESBL and CMY genes to the human strains by means of conjugal transfer of resistance plasmids’ [130].

Table 3 summarises findings from these various European studies.

Table 3. Percentage of retail meat samples which were positive for ESBL *E. coli* , with number of positives and number of samples in brackets

Country	Year published	Chicken	Pork	Beef	Other
UK (home produced)	2008 (sampled in 2006)	1.6% (1/62)	-	-	-
UK (imported)	2008 & 2010	30% (71/237)	-	-	-
UK (unknown)	2008	17.5% (7/40)	-	-	-
Netherlands*	2011	100% (1/1)	0% (16)	5.9% (17)	-
Netherlands	2011	77% (68/80)	2% (1/57)	5% (5/85)	-
Netherlands	2012	100% (92/98) [†]	-	-	-
Denmark	2011	3.3% (4/121)	2% (3/153)	0.7% (1/142)	-
Spain	2010	67% (8/12)	25% (3/12)	8% (1/12)	58% (7/12) ^a
Spain	2008	57% (27/47)	0% (0/30)	0% (0/22)	58% (7/12) ^b
France*	2011	43% (15/35)	0% (0/1)	-	-
Germany*	2011	34% (50/149)	0.7% (1/142)	0% (0/27)	-

*Tested as imported meat in Denmark; ^a Turkey; ^b Rabbit; [†] included 32 of 38 organic chickens

¹⁰ AmpC genes make the bacteria resistant to 3rd generation cephalosporins, but unlike ESBL resistance, not to 4th generation cephalosporins. On the other hand, AmpC genes do make the bacteria resistant to the antibiotic cefoxitin, which ESBLs are sensitive to.

7. The link between human and animal ESBL *E. coli*

Until recently, there were relatively few reports in the scientific literature of ESBL *E. coli* in farm animals. This, however, probably didn't reflect an absence of a problem, but rather a lack of research [132]. In the last few years, however, the number of studies being published has increased significantly. What this research appears to show is that, while human antibiotic use is certainly involved in the spread of ESBL resistance, in some countries, such as the Netherlands, where the animal epidemic, particularly in poultry, is at a more advanced stage than it is in the UK, there is already strong evidence that farm animals are important reservoirs of human ESBL *E. coli* or of their resistance genes.

In the UK, on the other hand, the evidence is not as clear cut. This is mainly because a single dominant clone until recently accounted for the majority of UK infections, and this clone does not yet appear to be directly associated with farm animals in the UK, or in Europe. However, as we shall see, even in the UK there is evidence that farm animals could already be contributing to the human problem, and if the incidence of ESBL *E. coli* in British farm animals continues to increase, particularly in poultry and perhaps pigs, the evidence from abroad suggests that this will have a severe impact on human health.

ESBL resistance genes are carried on plasmids, small loops of DNA which can reproduce inside the bacteria, and then be transmitted to other bacteria, thus spreading the resistance. Since many of the ESBL plasmids appear to be particularly transmissible between bacteria, this is making the spread of resistance complex and dynamic, with new strains and plasmids emerging in human infections.

The presence of some resistance plasmids in British pigs and poultry, which are still rare in *E. coli* causing disease in humans in the UK, but have become common in some other countries, suggests that unless action is taken to prevent these *E. coli* spreading further in the animal population, they are likely to emerge in humans in the UK too.

7.1. One dominant ESBL clone but diversity now over 50% and increasing

There are 90 known different CTX-M ESBL enzymes which can be produced by resistant *E. coli*, and scientists give each enzyme a number: CTX-M-1, CTX-M-2, etc [133]. When CTX-M ESBLs first emerged in *E. coli* the UK, a study carried out by the Health Protection Agency (HPA) found that approximately 90% of all cases in humans produced the CTX-M-15 enzyme. Furthermore, using a method known as pulse field gel electrophoresis (PFGE), the scientists showed that 65% of all cases were accounted for by just five dominant strains of *E. coli* producing the CTX-M-15 enzyme [88]. However, subsequent analysis of the five strains using a different method called multi-locus sequence typing (MLST) showed that the five strains were closely related and could all be viewed as a single clone, classified as sequence type ST131 [134].

This ST131 clone has since been shown to be widespread around the world [135][136][137]. Scientists are still unsure where it originated, but various indirect pieces of evidence point to the Indian subcontinent as a possible initial source. It has been shown that the CTX-M-15 enzyme was widespread in India prior to 2000, and the first known case of CTX-M-15 *E. coli* in the UK was in an Indian woman who had visited India in 1999 [138]. Studies carried out in New Zealand and Canada found higher levels of CTX-M-15 ESBL *E. coli* infections in people who had recently travelled to India or to the Indian subcontinent [139][140].

A Defra study published last September has found the first evidence that ST131 producing CTX-M-15 may be present in farm animals [141]. Other studies have shown that CTX-M-15 is one of the most common ESBL types in British cattle, chicken and turkey, but none of the strains producing the enzyme were ST131

[111][119][141][142]. Similarly, while the ST131 strain had previously been found in poultry in various countries, in some cases as ESBL-resistant strains producing the CTX-M-9 enzyme, they had not been found to produce the CTX-M-15 enzyme [78][141][143][144]. The Defra study, however, found an ST131 ESBL *E. coli* from a cow which produced either the CTX-M-15 or the CTX-M-28 enzyme. The CTX-M-15 and CTX-M-28 resistance genes are very closely related, and the Defra study did not distinguish between the two. However, CTX-M-15 is much more common in cattle and humans in the UK, so it seems likely that this isolate was CTX-M-15 [141].

When the isolate was analysed by PFGE, it was different to the main ST131 clones present in humans [141]. However, PFGE is a very sensitive typing method, highly valued for understanding outbreaks, but not so suited to longer-term epidemiology. As one HPA scientist explains: 'PFGE is comparative, rather than definitive, with patterns prone to change through mutation, DNA transfer and rearrangement events; such events may hide fundamental relatedness. Multilocus sequence typing frequently offers a more fundamental perspective of the population biology of a species' [145]. So, the finding of an ESBL ST131 *E. coli*, apparently producing CTX-M-15, in British cattle remains significant, despite the differences in PFGE. Nevertheless, this is the only case so far found in British farm animals and, even considering the small number of animal cases tested, it seems that ESBL ST131 in the UK are primarily circulating in humans rather than being acquired from farm animals. Further studies are clearly required to determine the extent to which farm animals may be implicated in this epidemic.

Although earlier British studies had shown that ST131 and the CTX-M-15 enzyme were overwhelmingly dominant in human ESBLs [88][146], there is now evidence of a greater diversity of strains of ESBL *E. coli* causing infections in humans. The most recent HPA research published last December shows that just under 50% of all CTX-M ESBL *E. coli* (and just under 45% of all ESBL *E. coli*) is now ST131 producing CTX-M-15 [147], a decline from 65% a few years earlier [88]. Approximately another 25% is made up of other strains producing CTX-M-15, and the final approximate 25% includes strains producing other enzymes, such as CTX-M-3, CTX-M14 and CTX-M-27. The study, which was based on strains collected in 2008-9, showed that at most 78% of the CTX-M were CTX-M-15, down from previous HPA studies which had found 85% for strains collected between 2003 and 2006 [148], and about 90% for strains collected in 2003-4 [88]. Other CTX-M which have been found in British human *E. coli* include CTX-M-2, CTX-M-9 and CTX-M-40 [148]. Many other CTX-M types have also been found in human *E. coli* abroad, including CTX-M-1, CTX-M-5, CTX-M-10, CTX-M-32, CTX-M-35 and CTX-M-61 [136][149][150][151].

The enzymes being produced by ST131 are also now more diverse: whereas in 2003-4, the only ESBL enzyme found in human ST131 in the UK was CTX-M-15, later studies have also found CTX-M-3, CTX-M-14 and CTX-M-27 in British cases, and in cases found abroad ST131 has also produced CTX-M-1, CTX-M-2, CTX-M-9, CTX-M-10, CTX-M-32, CTX-M-35 and CTX-M-61 [104][136][143][147][151].

Studies have also shown that the same gene for a particular CTX-M enzyme can be carried on different plasmids [136][152]. An example of this is the CTX-M-15 gene, which can be carried on several different plasmids in ST131 [136].

This diversity of CTX-M enzymes, of plasmids and of strains is significant as it shows that CTX-M ESBL resistance is not just spreading through what scientists call 'clonal dissemination', when one existing resistant strain is selected for by antibiotic use. The diversity shows that ESBL resistance is also spreading by the horizontal transfer of numerous resistance plasmids into new strains [110][152][153].

Because of this, many scientists are very concerned about the growing reservoir of ESBL genes in farm animals and warn they have the potential to transfer to strains which infect humans [3][59][109][124][126][133][143][144][152][154].

7.2. Evidence from abroad that human ESBL *E. coli* can be of farm-animal origin

As the number of studies in animals increases, evidence that ESBL resistance is transferring from farm animals to humans grows. Some of the most compelling evidence comes from abroad:

- Dutch scientists compared ESBL *E. coli* from retail poultry meat, poultry, and humans (including ESBLs other than the CTX-M type). They found a very high proportion (94%) of retail poultry meat had ESBL *E. coli*, and 39% of this belonged to *E. coli* strains also present in human infections. Of 514 human clinical cases of ESBL *E. coli*, 35% contained ESBL genes and 19% had resistance plasmids which were genetically indistinguishable from those found in poultry and in poultry meat. The scientists, including some Dutch government scientists, said 'These findings are suggestive for transmission of ESBL producing *E. coli* from poultry to humans, most likely through the food chain' [126]. One Dutch government scientist has said that the finding of these extremely high levels of contamination of Dutch poultry with ESBL *E. coli* 'strongly suggests that poultry products are the source for humans [of ESBL *E. coli*]' [155]. Another Dutch study found that 79.8% of retail poultry meat had ESBL genes and that the predominant ESBL genes in poultry meat and in human rectal samples were identical [125]. Furthermore, the *E. coli* strains in meat and humans showed a high degree of similarity. The authors concluded that 'these findings suggest that the abundant presence of ESBL genes in the food chain may have a profound effect on future treatment options for a wide range of infections caused by gram-negative bacteria¹¹'.
- A Japanese study found that 60% of live poultry were carriers of ESBL *E. coli*, and the ESBL gene types were all already found in Japanese human clinical cases. The scientists said that 'Mobile drug-resistance genes are capable of crossing bacterial species and are likely to accelerate dissemination of drug-resistance between animals and humans through chicken meat' [156].
- A study of ESBL *E. coli* from healthy Italian poultry found that most lacked the virulence genes required to cause infection in humans. However, 44% of the isolates were from MLST sequence types which had previously been found in humans. The scientists said that this suggested that these strains, particularly those of sequence type ST10, would be likely to be able to colonise humans, and transfer their resistance genes to other pathogenic *E. coli* in the human gut [157].
- A study has shown that poultry workers in the Netherlands have a much higher incidence of ESBL *E. coli* in their gut than members of the general population (30% versus 6%) [133]. The European Food Safety Authority has commented that this 'suggests that direct transmission from poultry to humans may also be a possible route of transmission' [133].
- A study on two Danish pig farms has similarly found CTX-M-1-producing *E. coli* from three of five farm workers, from 56 of 70 pigs and from manure and air samples [59]. Although there were numerous strains involved, the CTX-M-1 resistance gene found in the three workers from one of the farms was carried on a plasmid which was identical to the plasmids found in most of the pigs on the same farm. The scientists said 'This study illustrates that plasmids carrying ESBL genes of clinical interest can be easily transferred between animals and humans by direct contact. The high genetic diversity indicated that the spread of *bla*CTX-M-1 [the resistance gene] was not a result of clonal dissemination. Interestingly, even sows and piglets harbored distinct strains

¹¹ All bacteria are classified as Gram-positive or Gram-negative. *E. coli*, like salmonella, are Gram-negative.

despite being housed together. The same PFGE pattern was occasionally observed in *E. coli* of porcine and human origin, suggesting that some strains may be able to exist in the intestinal tracts of both pigs and humans, thereby allowing plasmid transfer'. Since the three farms workers carrying the ESBL *E. coli* had not received antibiotics or been in contact with hospitals in the previous six months, the scientists concluded 'Therefore, it is reasonable to assume that the farm workers acquired IncN plasmids carrying *bla*CTX-M-1 from pigs, where the presence of such plasmids was selected by antibiotic exposure'.

- Many of the plasmids found in ESBL *E. coli* from farm animals, food and humans are identical or closely related. According the European Food Safety Authority (EFSA) 'Epidemic plasmids belonging to incompatibility groups F, A/C, N, HI2, I1 and K carrying particular ESBL (TEM-52, CTX-M-1, -32, -9, -14) or AmpC (CMY-2) genes¹² have been detected among farm and companion animals, food products and human' [133]. In particular, EFSA say 'Highly transmissible epidemic IncFII plasmids carrying *bla*CTX-M-15 are of particular interest as they are globally-spread among *E. coli* and *K. pneumoniae* populations from humans and animals'.

For ESBL *E. coli* carrying the CTX-M-1 resistance gene, EFSA says that the similarities between the animal and human plasmids 'strongly suggests an animal reservoir for this ESBL gene variant, since either IncN or IncI1 plasmid types have been demonstrated to be highly prevalent in *E. coli* of the avian faecal flora and in *Salmonella* from retail meat and food-producing animals. These IncI1 and IncN plasmids are highly related to those in the community and hospitals' [133].

This is very significant since CTX-M-1 ESBL *E. coli* is very common in humans in some countries [125][158]. In Italy and the Netherlands, for example, where CTX-M-1 is one of the most common types of ESBL *E. coli* resistance in humans, CTX-M-1 ESBL are also found in poultry [125][153][125][159]. Italian scientists who found CTX-M-1 in poultry, as well as other ESBL types found in humans, have said that 'The similarity between the ESBL variants we detected and those present in humans in Italy indicates a possible role of poultry in the dissemination of these resistance determinants' [159].

- An increasing number of *E. coli* strains carrying ESBL genes are being found in both humans and animals or meat. This includes the strains classified by MLST typing as: ST4, ST10, ST23, ST48, ST57, ST69, ST88, ST93, ST131, ST155, ST359, ST354, ST393, ST398 and ST648 [125] [126][133][137][142][151][157][160][161].
- Circumstantial evidence links ESBL resistance with food. Spanish scientists studied ten outbreaks of gastroenteritis in Barcelona in 2003-4 where at least two of the individuals involved were found to be carrying ESBL *E. coli*. Food handlers and diners were investigated. In nine out ten of these outbreaks, at least two diners were carrying the same ESBL *E. coli* strain with the same resistance type, and in four of these food handlers were found to be carrying the same ESBL *E. coli* strain. The scientists were not able to test the food involved but concluded that their study provided 'circumstantial evidence that foods are a transmission vector for ESBL pathogenic bacteria' [131]. Another Spanish study found ESBL bacteria in faecal samples from patients in 19 of 61 food-borne disease outbreaks [100]. The prevalence of ESBL carriage ranged from 4.4% of people to 66%, according to the outbreak. The scientists said that their findings 'reinforce the hypothesis that ESBL-producing *Enterobacteriaceae*¹³ could be transmitted via the food supply'.

¹² AmpC resistance is a similar resistance to ESBL resistance. AmpC genes make the bacteria resistant to 3rd generation cephalosporins, but unlike ESBL resistance, not to 4th generation cephalosporins. On the other hand, AmpC genes do make the bacteria resistant to the antibiotic cefoxitin which ESBLs are sensitive to.

¹³ *Enterobacteriaceae* are a family of bacteria which includes *E. coli* and *Salmonella*.

- Scientists have reported that ‘In Denmark, the prevalence of ESBL-producing bacteria in food animals has increased over the past years, followed by an increasing prevalence of ESBL-producing bacteria in humans’ [47].
- A study carried out at an American hospital found that just 13% of patients who acquired ESBL *E. coli* while in hospital had acquired it from other patients. The authors concluded that ‘patient-to-patient transmission is not an important cause of the acquisition of ESBL-producing *E. coli* colonization in the intensive-care unit setting’ [162]. In contrast, many of the same scientists found that for another bacterial infection, *Klebsiella*, most resistant strains (53%) acquired in hospital were acquired from person-to-person transmission [163]. Since, even in hospitals, most ESBL *E. coli* do not appear to be acquired from person-to-person transmission, the likelihood that food is involved increases.

7.3. Can human ESBL *E. coli* be of farm-animal origin in the UK too?

In the UK, a number of studies have found links, as well as differences, between the ESBL *E. coli* found in farm animals and in humans.

A study by the Veterinary Laboratories Agency (VLA) compared virulence genes¹⁴ in ESBL *E. coli* from chickens and humans and concluded that ESBL *E. coli* which cause disease in poultry are generally different from those that cause disease in humans, partly because six of the ten virulence genes found in the human isolates were not found in any of the poultry isolates [164]. However, only two of the 29 poultry isolates included in the study had definitely caused disease in the birds. Had a larger number of definitely pathogenic poultry isolates been included in the study, it seems likely that some of the virulence genes found only in humans may also have been found in poultry. The VLA scientists point out that an Italian study had found one of the six human-only virulence genes in poultry [165]. Furthermore, we have found that at least two American studies, which examined much larger numbers of poultry isolates than the VLA study, also found this virulence gene, as well as two other of the virulence genes which the VLA had not found in their poultry isolates [166][167]. These two American studies did not look for the last three virulence genes which the VLA had only found in their human cases, but there are studies which have found one of these genes in *E. coli* from cattle, pigs and meat [168][169].

The VLA scientists conclude that ‘most chicken strains in GB are probably not directly associated with disease in people. This is different from what has been observed in Netherlands, where 35% of human clinical isolates contained what were considered poultry associated ESBL genes’ [164]. This appears to imply that in the Netherlands most chicken strains are probably directly associated with disease in people. However, the study they cite does not come to this conclusion. It finds that just 39% of retail poultry-meat *E. coli* were strains found in humans [126]. Despite this, the Dutch scientists found that poultry meat was still a major reservoir of resistance genes for *E. coli* infecting humans.

The VLA scientists say that ‘Of particular interest were the differences in the virulence genes carried by most (6/10) of the human isolates as compared to isolates from chickens’. However, the data they present shows that only five of the ten human samples had virulence genes not found in poultry, not six as stated in the paper. Given the very small sample size, this does not seem to be a compelling reason for downplaying the potential importance of ESBL *E. coli* in poultry. Several other studies, using much larger sample sizes, which have compared virulence genes from human *E. coli* and poultry *E. coli* have found considerable overlap [166][167][170], and a recent review has concluded that there is now mounting evidence that certain ‘clonal groups are capable of causing both human and animal infection’ [6]. The

¹⁴ Virulence genes are the genes which enable the *E. coli* to cause disease.

small VLA study does not present any evidence suggesting that this conclusion is likely to be any different for *E. coli* carrying ESBL genes.

Defra-funded research has also found that 'In relation to public health, a key finding is that within a defined geographical area, the CTX-M ESBL *E. coli* isolates from humans and those from cattle and sheep were unrelated and clustered into two separate groups at the level of virulence and antibiotic resistance genes' [111]. The precise significance of this is not clear. While the scientists suggest this is evidence for a lack of relatedness, one would not necessarily expect to find a local association given the fact that most food today is distributed nationally by multiple retailers.

However one views these findings, there remains a significant amount of evidence that ESBLs in British farm animals are contributing to the ESBL problem in humans, and may become an even greater part of it in the future. One British study, co-authored by VLA scientists, has said 'The ability and frequency with which antimicrobial resistance genes disseminate between bacteria in humans, the environment, and animals is still debated, and the role of plasmids in this movement between ecosystems, including the food chain, is also still contested, despite mounting evidence that it occurs' [171].

Some of the evidence that ESBL bacteria and genes are being exchanged between farm animals and humans in the UK includes:

- Data from VLA research show that most of the ESBL strains found in British poultry also cause infections in humans. This is in spite of VLA scientists claiming that their study had shown this wasn't the case. The VLA said: 'On the basis of MLST results, most of the isolates would appear to be different from human MLSTs, suggesting that the isolates identified as carrying CTX-M are probably not commonly transmitted to man from chickens or turkeys' [142]. However, the information included in their paper shows that 11 of 20 (55%) poultry isolates and five of ten turkey (50%) isolates tested had identical MLST types as ESBL cases found in humans [126]. Furthermore, information published since the VLA study show that another MLST type (ST57) found in poultry is now causing ESBL infections in humans [126], so 13 of the 20 (65%) poultry isolates were MLST types found in humans. The MLST types found in common between British poultry and turkey on the one hand and humans on the other include: ST4, ST10, ST57, ST88, ST155, ST156 and ST359.
- There is much less published information about the MLST types found in ESBLs from cattle. However, the epidemic human MLST, ST131, has been found in British cattle [141].
- Scientists from Birmingham University and the VLA studied a CTX-M-14 plasmid from *E. coli* taken from cattle. They developed a test, called a PCR (polymerase chain reaction) test, which enabled them to look for identical or similar plasmids in other *E. coli*. They found such plasmids in other UK farms and in UK human clinical cases. They also found similar or identical plasmids from Spanish human cases. They pointed out that in Spain CTX-M-14 is one of the most common ESBLs found in humans and animals, and that the most common Spanish CTX-M-14 plasmid appeared to be the same as the British one. This is of concern as it suggests that the prevalence of this type of resistance in the UK could increase in future years. The authors concluded that their findings showed that 'These data show the ability of a large conjugative plasmid to transfer between bacteria isolated from humans and animals, facilitating the movement of *bla*CTX-M-14 between these niches' [171].
- The most common CTX-M types in British cattle and turkeys are CTX-M-15 and CTX-M-14 [111][118][119][141][142]. In British chicken, the most common types are CTX-M-1 and CTX-M-15 [142], but CTX-M-14 has also been found [122]. This is similar to the types found in humans: in

the UK, CTX-M-15 is by far the most common type, followed by CTX-M-14 [147], and internationally the most common types are CTX-M-1, CTX-M-14 and CTX-M-15 [158]. Although poultry-origin CTX-M-1 ESBL have not yet become a major medical problem in the UK as they have in other countries, such as the Netherlands, this is probably because the levels of ESBL *E. coli* in UK poultry are lower. If, however, excessive antibiotic use results in increasing prevalence of these bacteria in poultry in the future, then there is every reason to expect that they will be transmitted to humans in increasing numbers.

- VLA studies have confirmed that several of the plasmids found in ESBLs from cattle and poultry are readily transmissible between *E. coli* strains [109][141][142] [171][172]. No study of the plasmids in pigs has been carried out, but in poultry and cattle the same plasmid has been found in different strains, showing that the plasmid was moving between strains [142][172]. A study on a cattle farm even showed that when the same plasmid was found in *E. coli* from a cow and her calf, it was usually in different strains [172]. Such readily transmissible plasmids pose a risk to human health, as they may transfer to *E. coli* in the human gut, which then cause infections.
- A survey carried out at a hospital in Wales found that 13% of the CTX-M ESBL *E. coli* infections were CTX-M-14, a prevalence that was higher than in earlier studies carried out elsewhere in the UK. The minutes of a Defra scientific advisory committee said that this was 'not common and rings an alarm bell' [173], presumably because CTX-M-14 is common in cattle in Wales.

8. Could farm antibiotic use be contributing to the increase in *E. coli* blood poisoning in humans?

In the hope of discovering why the number of *E. coli* blood-poisoning infections continues to increase, the government introduced mandatory surveillance for such infections in June 2011 [18]. One factor which is likely to be important is that we have an aging population: between 1985 and 2010, the number of people aged 65 and over in the UK increased by 20%, and the number aged 85 and over doubled [173]. This may be significant since the risk of an *E. coli* infection increases with age (see Graph 2). Increased levels of *E. coli* in the human gut have also been linked to excessive weight gain in pregnant women [175]. Higher levels of obesity could, therefore, be having an impact too, perhaps specifically in relation to meningitis in newborn babies.

8.1. Studies link antibiotic feed additives with increasing *E. coli* levels

Farming practices may also be having an effect on the number of *E. coli* infections. The number of pathogenic *E. coli* entering the human gut on food is likely to be proportional to the number of *E. coli* bacteria on meat and other foods. This in turn will relate to the levels of *E. coli* in farm animals, and the extent to which these are able to transfer to and survive on livestock carcasses at the abattoir, as well as contaminate other foods through the environment. Recent research from the United States suggests that it is possible that the use of antibiotics on farms is a factor contributing to high levels of *E. coli* in farm animals.

Research published in January by scientists from Michigan State University and the US Department of Agriculture's Agricultural Research Service shows that populations of *E. coli* in pigs on medicated feed can be very much higher than those in non-medicated pigs [178]. Dr Thad Stanton, one of the microbiologists involved said, 'We were so surprised that we did a repeat experiment in culture. And the number went up by 20 to 100-fold. That's a big change' [179].

The antibiotic combination used in their trial, chlortetracycline, a sulphonamide and penicillin was once widely used in pig feed as a growth promoter in the UK, but its use ceased in the early 1970s, following recommendations from the Swann Committee report in 1969 [180]. As such, this study is not directly relevant to the situation in the EU where the use of antibiotics for growth promotion is prohibited. However, all three antibiotics are still available singly, and are sometimes prescribed by vets in combination [181]. In 2010, these three antibiotics together accounted for 82% of all veterinary antibiotics sold in the UK – the tetracyclines, beta-lactams (penicillin-type antibiotics) and sulphonamide antimicrobials accounting for 45%, 21% and 17%, respectively, of antibiotics used in livestock production [182]. In addition, chlortetracycline remains the most widely used antibiotic in pig feed.

The Michigan study confirms findings from a small number of other studies, the first of which was carried out over half a century ago. In 1953, scientists at the University of Georgia in the US found that very low levels of penicillin in pig feed increased the population of coliform bacteria in pig faeces 100-fold [183] – *E. coli* are the predominant coliform bacteria in pig faeces [184]. In 1978, scientists from the University of Kentucky found that feeding the antibiotic virginiamycin to pigs, used as a growth promoter in the UK until 1999 and still used in the United States, caused statistically significant increases in coliforms in their faeces [185], and in 1984 the same group of scientists used a marked inoculated strain of antibiotic-resistant *E. coli* to measure the effect of in-feed antibiotics specifically on *E. coli*. They found that pigs given chlortetracycline in their feed had almost 100,000 marked *E. coli* bacteria per gram of faeces after

25 days, when by then the non-medicated pigs had none [186]. It has also been reported that including the antibiotic tylosin in pig feed, which is still used as a feed additive in pig feed (although since 1999 it is no longer licensed as a growth promoter in the EU), led to 'a slight elevation in the number of *E. coli* per gramme of faeces from treated pigs' [187].

There has long been a theoretical case that farm animals given certain antimicrobials in feed or water will have higher levels of some bacteria, such as *E. coli* and *Salmonella*. This is because some antibiotics only kill certain types of bacteria, leaving other types unharmed, with more nutrients available and room to grow.

All bacteria are divided into two groups: Gram-positive bacteria and Gram-negative bacteria, according to whether they appear purple or pink after laboratory staining. The different staining is not cosmetic, it arises due to the very different nature of the cell wall in two types of bacteria, and as a result certain antibiotics only kill Gram-positive bacteria, others only kill Gram-negative bacteria and those that kill both are usually described as 'broad-spectrum'.

More of the antibiotics added to pig or poultry feed have a predominantly Gram-positive action (e.g. penicillin and tylosin¹⁵) than Gram-negative (e.g. streptomycin), and these Gram-positive antibiotics can be expected to favour *E. coli* growth because they kill off or suppress a proportion of the bacteria with which *E. coli* are in competition but do not directly affect the *E. coli*, whereas the Gram-negatives will often inhibit such growth.

VLA research published in 2006 also found a possible link between the use of Gram-positive growth promoters and the levels of antibiotic-resistant *E. coli* from pig and poultry farms. The study involved 13 non-organic pig and poultry farms (which were still at the time using growth promoters) and 12 organic farms. It found much lower levels of antibiotic use on the organic farms. In addition, it found that the levels of resistance to the beta-lactam antibiotic ampicillin in *E. coli* on the non-organic farms were not correlated, as one might expect, with beta-lactam antibiotic use. Instead, there was a statistically significant correlation with the use of the Gram-positive growth promoter avilamycin, which at the time was the most widely used antibiotic on non-organic pig and poultry farms.

The scientists said : 'the hypothesis that the application of avilamycin may promote the detection of AR *E. coli* is an interesting concept as avilamycin does not exert direct effects upon gram-negative organisms such as *E. coli*. However, the administration of low doses of growth promoters suppresses certain members of the gut flora, and thereby decreases factors such as nutrient competition, digestive enzyme breakdown and gut wall damage, thus enhancing the growth of the host animal. Therefore could it be that alterations in the balance of gut flora may indirectly affect the numbers of resistant bacteria by reducing the numbers and/or variety of important competing species?'. They suggested that this possibility merited 'further attention' [188]. Avilamycin was only licensed as an antibiotic growth promoter, not a therapeutic antibiotic, and it has not been used in the EU since 1 January 2006, when all use of growth-promoting antibiotics ended. It is therefore unlikely that further research has been initiated, as recommended by the study's authors, even though the phenomenon applies to other antibiotics that are still in widespread use.

¹⁵ Most of the formerly licensed growth promoters were Gram-positive, and some of these (eg. tylosin, monensin and salinomycin) can still be used in animal feed, under veterinary prescription. Gram-positive growth promoters no longer on the market in the EU include eg. avilamycin, avoparcin, bacitracin, flavomycin, virginiamycin

The effect of broad-spectrum antibiotics on *E. coli* numbers is likely to be more complicated since, in theory at least, they inhibit the growth of both *E. coli* and their competitors. The Michigan research shows that, despite this, a feed additive containing chlortetracyclines, a broad-spectrum antibiotic, greatly increased *E. coli* prevalence.

8.2. Resistance can influence effect of antibiotics on *E. coli* numbers in farm animals

For every study, however, that found increased numbers of *E. coli* in farm animals given antibiotics in their feed, there are at least as many finding either no effect, or in some cases an actual reduction. There is a similar lack of consensus among the more than 50 studies that have taken a look at the impact of medicated feed on the levels of salmonella in farm animals.

For many years one rather obvious explanation for this was not generally recognised. And the explanation for why apparently similar studies produced such very different results also helps to explain why the *E. coli* levels in many intensively farmed animals today are likely to be at an all time high.

In the late 1940s the first broad-spectrum antibiotics, the tetracyclines were developed. These are equally effective against both Gram-positive and Gram-negative bacteria. Apart from coming into use as life-saving antibiotics for both humans and animals, the tetracyclines were used for growth promotion in the US almost immediately. In the UK they were used on seven experimental farms in 1950, and from 1953 more widely on intensive livestock farms. An account of what happened in relation to the tetracyclines will serve to explain one of the reasons why not all the studies undertaken found higher levels of *Salmonella* in chickens given in-feed growth promoters and also to illustrate a phenomenon that is likely to be occurring now in relation to both *Salmonella* and *E. coli* with a wide range of antibiotics.

Initially, in the early 1950s, when chlortetracycline was first licensed for regular use in pig and poultry feed and calf milk, nearly all *E. coli* would have been sensitive to the antibiotic, as would most of the Gram-positive bacteria in the gut, like the beneficial lactobacilli. As such, the overall impact of chlortetracycline would have been minimal as it would have had a broadly equal impact on both Gram-positive and Gram-negative bacteria, which is what most early studies show.

So while the recent Michigan study found that pigs fed feed containing chlortetracycline and other antibiotics had a very significant increase in the prevalence of *E. coli* in their gut, a study which examined the effect of tetracyclines on pigs that had never previously been exposed to antibiotics found a small, but not statistically significant reduction in the number of *E. coli* [185]. An earlier study, published in 1950, found no effect from tetracyclines on the prevalence of *E. coli* in pigs [189], probably because at this point when the tetracyclines were only just starting to become available most, or all, of the *E. coli* strains would have been sensitive to them.

However, within a few years, a proportion of the many pathogenic strains of *E. coli* in animals receiving antibiotics regularly began to develop resistance to the tetracyclines and other antibiotics, and this progressively increased. By 1971, scientists in the US, who collected 55 different strains of *E. coli* from animals on five farms, found that 84.8% of these were resistant to antibiotics on farms where antibiotics were widely used. In contrast on a farm where animals were not given antibiotics, only 15.7% were resistant [190]. VLA data show that by 2007 in the UK, 78% of *E. coli* from pigs were resistant to tetracyclines, as were 75% of *E. coli* from cattle, 67% from turkeys and 51% from chicken [194].

The Michigan research and the earlier Kentucky research suggest that the high levels of tetracycline resistance now present mean that the continuing high level use of tetracycline antibiotics in feed will

increase the total number of *E. coli* in the gut of farm animals. This, in turn, is likely to increase the level of carcass contamination and the possibility of transmission to humans, but is also likely to have an impact on animal health. *E. coli* causes 'huge losses' to the poultry industry as it causes 'colibacillosis' which usually begins as a respiratory infection and which may lead to blood poisoning and death [6]. As a result, it can be argued that overuse of tetracyclines in livestock production is increasing not decreasing livestock diseases of economic, as well as public-health, significance.

In 1998, Belgian scientists found that 69% of the pathogenic strains of *E. coli* that cause infections in commercial poultry were resistant to tetracyclines. They also found that this was causing serious economic losses with almost 40% of laying hens and up to 27% of broiler chickens affected by serious illness [192]. *E. coli* also causes scouring (diarrhoea), mastitis and urinary-tract infections in cattle and pigs.

Due to the progressive development of chlortetracycline resistance in *E. coli* in farm animals, it is now rarely used to treat infections caused by *E. coli* because it is no longer effective. Such infections generally occur in young animals. But it is still used when the animals are older and less at risk of *E. coli* infections. This may increase the numbers of *E. coli* in the gut, and since this will be nearer to the time when they are slaughtered, it will also increase the likelihood of high levels of *E. coli* on carcasses if faecal contamination occurs.

The high levels of resistance to older antibiotics explains why the use of more modern drugs, where resistance levels were initially much lower, has been increasing. However, as resistance develops, a very similar thing is now likely to be happening with these antibiotics too. The 3rd generation cephalosporin ceftiofur is given by injection and is not used as a feed additive, like chlortetracycline. However, 20-40% of it is nevertheless excreted through the gut in the faeces [193]. The fluoroquinolone antibiotic, enrofloxacin, however, can be given to both piglets and poultry orally. Since ESBL *E. coli* are resistant to a wide range of antibiotics, the use of both ceftiofur and enrofloxacin is likely to select for them once they are present in the animals' intestinal tract, because the broad-spectrum nature of these antibiotics means that they will kill off or suppress a high proportion of gut bacteria with which ESBL *E. coli* would normally have to compete.

The recent Michigan study brings back into the open important questions about the effect of antibiotic feed additives on both antibiotic resistance and the overall level of pathogens. In the UK this was the subject of active research by government scientists during the 1970s and early 1980s, but funding for this work ended abruptly, with the introduction of the concept of 'near-market funding', under which the government left it to drug companies to undertake all safety research in relation to antibiotics that were already on the market or being developed for it. The drug companies however, have had little incentive to undertake research in this area, except when it has been to counter independently funded scientific research suggesting that their products increased the incidence of *Salmonella*. Further independent research in this area is therefore urgently needed, as both the Michigan and VLA scientists have recommended.

An alternative to using antibiotics to suppress or kill *E. coli* infections in intensively reared animals is instead to encourage beneficial bacteria which compete with *E. coli* and limit their growth, as in nature. Products are also available that can be used at the same time as antibiotics to minimize their negative effects. Under natural conditions, baby chicks, piglets and calves will acquire a healthy natural gut microflora from their mothers, which will go a long way to prevent *E. coli* infections occurring. However, chicks reared in hatcheries and calves taken from their mother at birth have little opportunity to develop an ideal gut microflora. While piglets in theory have a slightly better opportunity, they are generally

weaned at 3–4 weeks from their mothers, still a month away from having a fully developed gut immune system, which is why a type of *E. coli*, more closely related to *E. coli* O157 than to ExPECs, frequently cause infection in piglets at weaning, and why many producers still routinely give them in-feed antibiotics at that time in expectation of this. There have been many studies looking at different aspects of this issue; we cite just one by way of an example [195].

8.3. Antibiotic resistance increases *E. coli*'s ability to reach humans through the food chain

Researchers at Bristol University have examined whether antibiotic-resistant *E. coli* survive better on meat carcasses than sensitive *E. coli*. They inoculated a number of pigs with an antibiotic-sensitive *E. coli* strain and others with a multiple antibiotic resistance (MAR) mutant of the original *E. coli* strain. They found that the MAR mutant *E. coli* were better able to withstand the slaughter and chilling process than the sensitive *E. coli*, thereby increasing their chances of passing along the food chain.

The scientists said that, 'The MAR derivatives were recovered from 11 of 13 abattoir locations sampled, the number of MAR derivatives was 10-fold higher than the parent strain at half these locations and the MAR derivatives persisted on 3 out of 5 chilled carcasses; in contrast the parent strain was recovered from only half as many sampling locations 6 out of 13 and it was not recovered from any of the 6 carcasses post chilling'. They acknowledged that their experiment was only with one particular strain of *E. coli* but nevertheless said that it showed that, for at least some bacteria, multiple-antibiotic resistance 'increased the likelihood of them passing along the food chain [to humans]' [196].

In addition, the use of antibiotics by humans is also likely to increase the problem in relation to antibiotic-resistant strains of *E. coli* coming from animals. In 1974 researchers showed that human volunteers taking therapeutic doses of tetracycline orally shed tetracycline resistant *E. coli* of bovine origin for longer than those not taking the antibiotic [197].

As such, while this aspect has been the subject of very little research in recent years, it can be seen that there are a number of reasons why the rising levels of antibiotic resistance in the *E. coli* strains in farm animals are likely to be increasing the chances of *E. coli* infections in humans, in addition to increasing the likelihood that those infections will be highly antibiotic resistant. It can also be seen that action to limit all non-essential use of antibiotics needs to be taken in both human and veterinary medicine.

9. Antibiotic use in farm animals is increasing

In addition to the studies considered in Chapter 8 which show that the use of some antibiotics can increase the overall number of *E. coli* bacteria in animal faeces, numerous studies carried out over many years have shown that including antibiotics in animal feed also increases the proportion of antibiotic-resistant *E. coli* in the animals' intestines. [185][191][198][199][200][201][202][203].

Of particular concern is that this relationship between antibiotic use and high levels of resistance in *E. coli* has also been established for the two antibiotic families classified by the World Health Organization as 'critically important in human medicine', the fluoroquinolones and the modern cephalosporins.

Scientists have linked the use of fluoroquinolones in farming to high levels of resistance in *E. coli* in both pigs and poultry [71][204][205]. It has also been established that the use of antibiotics, particularly the modern cephalosporins, can increase the prevalence of ESBL *E. coli* in farm animals. An experiment carried out by Danish scientists showed that treating pigs (previously inoculated with a strain of ESBL *E. coli*) with the beta-lactam antibiotic amoxicillin, increased the quantity of the ESBL bacteria in the animals' faeces compared with untreated pigs, and that treating the pigs with the modern cephalosporins ceftiofur and cefquinome increased their prevalence even more [206]. Two other studies of Danish pig farms have found a statistically significant association between cephalosporin use on pig farms and the prevalence of ESBL *E. coli* in pigs from the farm [207][128].

British research has also shown a link between the farm use of modern cephalosporins and ESBL *E. coli*. A VLA study of 65 cattle farms found that those that had used modern cephalosporins in the previous year were four times more likely to test positive for ESBL *E. coli* [117].

ESBL *E. coli* also frequently carry resistance genes to many other classes of antibiotics on their ESBL plasmids. This can include, for example, genes for resistance to the fluoroquinolones, aminoglycosides, sulphonamides, tetracyclines and trimethoprim [10][208]. Consequently, use of any of these classes of antibiotics can be expected to 'co-select' for ESBL resistance [10]. This is already known to occur in human medicine [208], and scientists expect that it will also be occurring with farm animals [119]. The European Food Safety Authority has said that 'resistance to several antibiotics implies that once ESBL – or AmpC – producing isolates have entered a production unit, a broad range of antimicrobials can favour their selection and spread between animals' [133]. The issue of ESBL *E. coli* being co-selected for in farm animals by non-cephalosporin antibiotics has recently been investigated by the Animal Health and Veterinary Laboratories Agency (AHVLA), but their results have not yet been published [209].

In 2000, the then government accepted a recommendation from its Advisory Committee on the Microbiological Safety of Food (ACMSF) that the government should develop 'a coherent strategy aimed at reducing the veterinary use of antibiotics' [210][211].

However, despite initial encouraging signs, when the British government joined forces with other European countries to implement an EU-wide ban on the antibiotic growth promoters, there has been no conviction to the policy. Very little has been done by the previous or the current administrations to discourage farmers from simply increasing their use of antibiotics in feed or water for 'disease prevention'. One of the incentives for doing this is that it continues to provide the growth-promoting benefits of low level in-feed antibiotic usage, but under the guise of disease prevention, as a number of antibiotics formerly used for growth promotion are still authorised for use prophylactically as well as therapeutically. Growth-promoting doses are generally lower than prophylactic doses, which in turn are

lower than therapeutic doses given to treat sick animals. However, the doses for prophylaxis and growth promotion can overlap, and for some licensed antibiotics the low prophylactic doses are the same as the doses formerly used for growth promotion.

There is little publicly available information on the way in which antibiotics are currently being prescribed for use on livestock farms in the UK. There also appears to be no oversight of the choices made by veterinary surgeons when selecting antimicrobials, dose levels and duration of use. As such we do not know the extent to which this varies between prescribing veterinarians or the extent to which it may vary between independent veterinarians and those employed directly by large intensive livestock companies.

To the extent this area has been monitored at all, it traditionally fell to the Animal Medicines Inspectorate of the Royal Pharmaceutical Society of Great Britain. But in 2006 responsibility was passed to the Veterinary Medicines Directorate's Inspection and Investigations Team [212]. The team has a wide range of responsibilities, but is not clear that they ever give informed consideration to the prescribing patterns of individual veterinary surgeons and/or the level of antibiotic use on different farms as now happens in Denmark. Also, their responsibilities do not include monitoring the use of antibiotics on farms with a view to minimizing the development and spread of antibiotic resistance. This is probably because regulation of farm antimicrobial use in the UK is still largely focused on ensuring that drug residues above permitted limits do not enter the food chain. This was a major concern in the past, but the mechanisms set up to address this were not designed to also minimise the development of antimicrobial resistance. Consequently, this is an area that requires significant attention in order to ensure that the regulation of antimicrobial is fit for the 21st Century.

EU Directive 90/167/EEC (currently being revised) lays down rules for the production of medicated livestock feed. On-farm incorporation of antibiotics is permitted under a derogation from Article 4(2) of the Directive. While some countries, such as Denmark, have not taken advantage of this derogation, in 2010 there were 640 licensed on farm operations in the UK where antibiotics could be included in livestock feed on-farm. Such feed mixers are not permitted to incorporate antibiotics at a rate of less than 2 kg per tonne [213]. The monitoring of such premises also falls to the Inspections and Investigations Team, but it is not clear that there is a sufficiently high level of inspection or any testing of feed to determine the actual level of antibiotic inclusion. In addition, the vast majority of prescribing for in-feed or in-water medication is done over the telephone without any checking even by the veterinary surgeon of the number of animals needing treatment. This suggests there is little to deter an unscrupulous producer from either requesting antibiotics from his vet for a smaller number of animals than he plans to feed with medicated feed, or mixing a prescribed dose of antibiotics with a larger amount of feed and therefore being able to feed antibiotics over a prolonged period of time at low, growth-promoting doses, in both cases without the knowledge or approval of a veterinary surgeon.

Twenty years ago, as a result of evidence linking the use of meat and bone meal in cattle rations with the BSE epidemic, an advisory committee, chaired by Professor Eric Lamming from Nottingham University, was set up to consider whether there were other potential problems associated with livestock feed. The committee's report, published in 1992, recognised that the use of antibiotics for disease prevention, as well as growth promotion, could have consequences for human health, and said: 'We recommend that not only should antibiotics giving cross-resistance to those used in human medicine not be used as growth-promoters but that their prophylactic use in animals be reconsidered' [214]. Successive administrations have, however, ignored the recommendation to reconsider prophylactic usage, and no formal consideration has ever been given to the subject in the UK. This, despite the government's own

scientists at the Central Public Health Laboratory regretting the lack of implementation of the recommendation [215].

9.1. Statistics for total antibiotic use in UK farming

The above factors may help to explain why total antibiotic consumption has fallen very little since 2000. The small decline that has occurred is due overwhelmingly to a reduction in pig numbers, rather than lower use per animal. Pigs account for approximately 60% of farm antibiotic use in the UK, and pig numbers have fallen by over 30% since 2000, and by over 45% since 1998 [216].

Since 1998, the Veterinary Medicines Directorate (VMD) has reported statistics on antibiotic sales in British farming [217], and in 2000 the government accepted a recommendation from the ACMSF that this should be broken down by species [211]. There is, unfortunately, no central record kept of antibiotic use in the UK and this has prevented actual usage each year being allocated to individual species. The VMD statistics are based on the sales data reported to them by pharmaceutical companies. When the VMD first began collecting this data, cooperation from the industry was on a voluntary basis, and as a result the statistics proved very unreliable, with major revisions sometimes occurring several years later [218]. However, from 2005 onwards, an EU directive has required statutory collecting of sales data from the drugs companies [219], and fewer revisions have occurred since. Table 4 provides the sales data from 1998 to 2010.

Table 4. Sales of antibiotics for use in farm animals, 1998 to 2010, including growth promoters (tonnes of active ingredient)

1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
513	423	424	393	406	395	407	395	358	335	327	349	390

The statistics appear to show a significant fall in comparison to 1998 at least, however we shall show that once we adjust for changes in livestock numbers in each species, and take into account the different consumption rates in different species, the rate of consumption per animal in 2010 was actually the highest ever.

The VMD now provides a partial breakdown of antibiotic consumption by species based on a limited survey. In 2010, this showed that an estimated 211 tonnes were sold for use in pigs, 138 tonnes were sold for poultry, 11 tonnes were sold for cattle, one tonne in fish and less than 0.5 tonnes were sold for use in sheep [182]. The remaining 29 tonnes were sold for use in 'multi-species'. In others word it wasn't possible to determine which species these antibiotics were being used for as they were licensed for several species.

Unfortunately, similar species breakdowns for earlier years are only available from 2007 onwards. Had a species breakdown been available for each year, it would have been straightforward to see if the amount of antibiotic being used per animal was going up or down in each species. We can, however, make such a comparison from 2007 onwards, and Table 5 shows that in 2010 consumption per animal in pigs and poultry (which together account for about 96% of farm antibiotic use) was at its highest level yet¹⁶.

¹⁶ We have not included cattle and sheep in Table 5 because the levels of consumption are so much lower than in pigs and poultry, and the VMD statistics not sufficiently accurate to make meaningful year-on-year comparisons (the statistics are given in tonnes of active ingredient, and statistics in kgs of active ingredient would be needed to make meaningful calculations for cattle and sheep).

Table 5. Use of antibiotic per animal for pigs and poultry, 2007 to 2010 (grammes of active ingredient per animal per year)

	2007	2008	2009	2010
Pigs	41.2	38	41.2	47.3
Poultry	0.602	0.656	0.766	0.842

In order to make a meaningful comparison of 2010 consumption levels with earlier years, which gives greater weighting to changes in the numbers of the main consuming species, pigs and poultry, than to the numbers of cattle and sheep, we need to make a more complicated calculation because statistics for consumption in each species are not available for earlier years. We will work through an example, where we compare consumption in 2010 with consumption in 1998.

For 2010, we have an estimate of the consumption for each species as well as an estimate for ‘multispecies’ consumption. If we assume that the ‘multispecies’ antibiotics were used in the different species at the same proportion as the other antibiotics, then for 2010 we can estimate that 228 tonnes were used in pigs, 149 tonnes were used in poultry, 12 tonnes were used in cattle, 1 tonne in fish and less than 0.5 tonnes in sheep.

For 1998 we do not have a species breakdown, however, we do have the numbers of animals in each species in 1998 and 2010 [216]. These show that compared with 2010, in 1998 there were 82.6% more pigs, 0.7% more poultry, 13.9% more cattle and 43.1% more sheep. We can now calculate what total farm consumption would have been in 1998 had animals in 1998 been consuming at the rate at which animals in their species were consuming in 2010¹⁷. We call this the ‘2010 rate’ for 1998.

Since there were 82.6% more pigs in 1998 than in 2010, the ‘2010 rate’ for pig consumption in 1998, for example, would be $228 \times 1.826 = 416$ tonnes. This is what total pig consumption in 1998 would have been if all the pigs were consuming at the rate at which they were consuming in 2010. Similarly, the ‘2010 rate’ for total farm consumption in 1998 is calculated as:

$$228 \times 1.826 + 149 \times 1.007 + 12 \times 1.139 + 0.5 \times 1.431 + 1 = 582 \text{ tonnes}$$

According to VMD statistics, actual reported consumption in 1998 was 513 tonnes, including the growth promoters. So we can see that if animals in 1998 had been consuming at 2010 rates, they would have been consuming more. In other words, the consumption in 2010 was higher than in 1998, once we take into account the changes in the number of animals in each species.

Similarly, we can calculate a ‘2010 rate’ for total antibiotic consumption in the years 1998 to 2009, and compare with the actual consumption reported by the VMD. Table 5 compares this ‘2010 rate’ for each year and shows that it is higher for all years between 1998 and 2009.

¹⁷ We assume constant fish numbers in this calculation, as no statistics on fish numbers are available. This will not materially affect the outcome of the calculation, as fish consumption only accounts for a very small proportion of total consumption.

Table 5. Total farm antibiotic consumption if consumption had been at 2010 levels per animal in each species, compared with actual antibiotic consumption, 1998 to 2009 (tonnes of active ingredient)

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
2010 rate	582	538	501	476	453	435	443	421	423	413	406	384
Actual	513	423	424	393	406	395	407	395	358	335	327	349

We can conclude from Table 5 that the rate of consumption in 2010 was higher than in any year between 1998 and 2009. The rate of consumption per animal since 2000, when the government announced its aim to reduce veterinary antibiotic consumption, has actually gone up by 18%.

The VMD has claimed that because farm productivity has increased, the rate of consumption has roughly fallen in line with the fall in the total live weight of animals slaughtered for food use [220]. However, while it is true that the average weight of a slaughter pig increased by 10% in the last ten years, and by 25% over the past 25 years [221], the VMD's argument fails to take into account the fact that since 1998 the overall live weight of slaughtered pigs, the largest antibiotic consumers, has fallen by over 35% since 1998, whereas the live weight of slaughtered poultry and cattle has increased and of slaughtered sheep has fallen by 20%. As a result, pigs now account for a much smaller percentage of the total live weight of slaughtered animals, and we would therefore have expected to have seen a much larger fall in the consumption of antibiotics per tonne of meat produced¹⁸.

Current usage also hides a further trend which indicates that the use of antibiotics in terms of the number of doses rather than the weight of active ingredient has increased even more: while still very high, usage of the very bulky tetracyclines has fallen recent years as the use of more modern and lighter antibiotics has increased, see section 9.2.

The recent ESBL report by the government's advisory committees DARC and ARHAI, considered what the consequences would be of increased restrictions on antibiotic use on British farms. They said this would lead to increased costs, either because there would be greater mortality or because 'livestock have to be kept more extensively or in better buildings to minimise risks of becoming infected, such as avoiding pneumonia by building better designed, well-ventilated buildings' [10]. There is an implicit recognition in this statement, that routine antibiotic use is what enables farmers to keep animals in highly intensive and unhealthy conditions. Yet, despite recognising that restrictions on antibiotic use are needed to reduce the level of ESBL *E. coli* in farm animals, and could have the desirable consequence of encouraging farmers to keep animals in more extensive and hygienic conditions, the government's committees advised against such a move, suggesting that it would lead to greater imports of produce produced under fewer restrictions.

This unwillingness to take action to reduce overall usage contrasts with many initiatives being taken in other EU countries. In the Netherlands, there is an aim to reduce antibiotic use by 20 per cent between

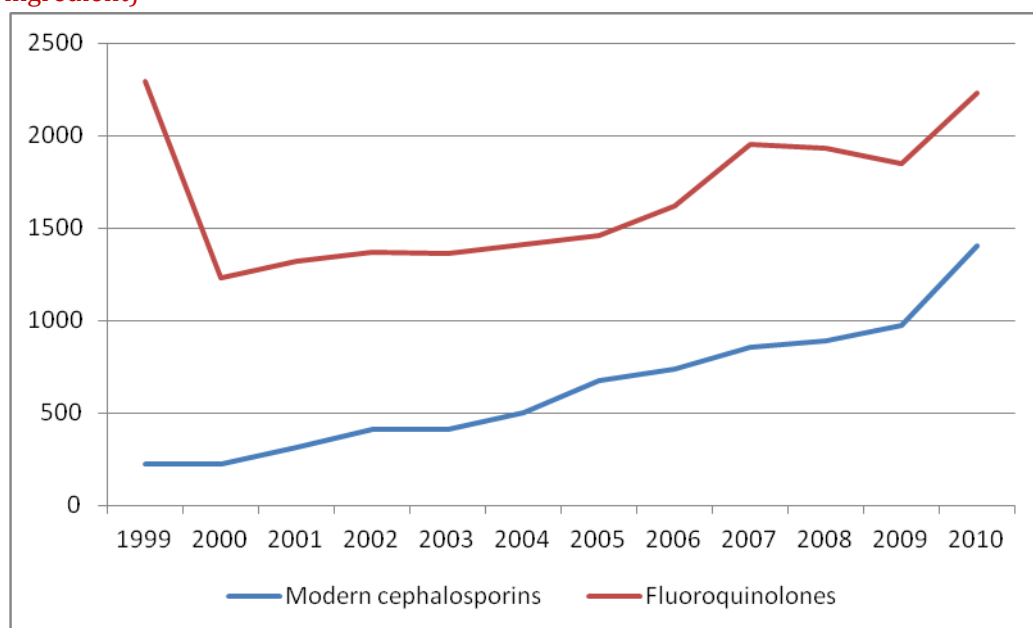
¹⁸ If we carry out a calculation with weight of animals slaughtered for human consumption, similar to the previous one above with livestock numbers, we find that the consumption rate in 2010 was the third highest since 1998, lower only than 2004 and 2005. However, this is a problematic comparison as it does not take into account the fall in the numbers of animals (which will increase slaughter weight in the short term), the increase in the numbers of live pigs being imported from the Republic of Ireland for slaughter in Northern Ireland, or the fall in the number of live animals exported over this period. It also doesn't take into account the effect of the removal of the Over Thirty Month Rule for slaughtering cattle for human consumption in November 2005.

December 2010 and 2011, and by 50 per cent by 2013. There are also plans to name and shame farmers who exceed a specific level. In Denmark, a new government monitoring scheme scrutinises the quantities of antibiotics used on farm. A yellow card system is in place for those who exceed a set level. In France, an action plan has been published which aims to reduce antibiotic usage by 25 per cent over the course of the next five years [82] [222]. In Germany, Agriculture Minister Ilse Aiger has proposed changes to German veterinary medicines legislation aimed at keeping antibiotic use to the minimum. These will include vets being compelled to give investigating authorities information about the antibiotics they are prescribing [223].

9.2. Large increases in the use of critically important antibiotics on British farms

For many years there have been annual increases in the UK in the farm use of fluoroquinolones and the modern cephalosporins. Graph 5 shows how, after a large fall in fluoroquinolone consumption in 2000, following a recommendation from a House of Lords select committee that this needed to be reduced [224], the farm use of both the families of antibiotics has increased nearly every year over the past decade¹⁹.

Graph 5. Veterinary use of modern cephalosporins and fluoroquinolones 1999 to 2010 (kgs of active ingredient)



Despite the ongoing increases, it may nevertheless appear that in terms of weight of active ingredient, the quantities of the modern cephalosporins and fluoroquinolones are still small compared to the consumption of other antibiotic classes. In 2010, for example, there were 1,400kg of modern cephalosporins and 2,232 kg of fluoroquinolones used in veterinary medicine, whereas the use of tetracyclines, the most widely used antibiotic on British farms, was 200 tonnes. However, a single dose of one of the modern cephalosporins or the fluoroquinolones weighs a lot less than a single dose of tetracyclines, so in terms of the number of doses, the disparity is not nearly as large. For example, a single dose of fluoroquinolone antibiotic typically weighs (depending on species) 30 to 70 times less than

¹⁹ Some of these statistics for Graph 5 come from the VMD annual sales data reports [225], others come from Freedom of Information requests submitted by the Soil Association to the VMD.

a single dose of tetracycline antibiotic [226]. These increases in consumption are therefore a genuine cause for concern.

Although these are not drugs of last resort in hospitals, they have been the main drugs of choice for a wide range of serious infections, including *E. coli* infections. The development of resistance to them therefore increases treatment problems in hospitals, increases costs to the NHS and increases the use of drugs of last resort which, in turn, increases the likelihood that resistance to them will also develop and spread. Sir Liam Donaldson, the former Chief Medical Officer, was so concerned about the irresponsible use of these antibiotics in farming that in 2009 he called for an outright ban on the use of fluoroquinolones and modern cephalosporins in animals [227]. Government scientists from the Veterinary Laboratories Agency and the Health Protection Agency have also expressed their concern, and have said 'Where possible, the use of newer generation cephalosporins should be limited in veterinary medicine' [228].

In November 2009, the British Veterinary Association published an 8-point plan for limiting the development of antibiotic resistance in farm animals [229]. One of its recommendations was that vets should keep the fluoroquinolones and modern cephalosporins in reserve and only use them in very limited situations, a recommendation very similar to Soil Association standards (see Section 9.3 below). But despite the BVA's plan, in 2010 the use of modern cephalosporins increased a further 43% over 2009 levels to an all-time high, and the use of fluoroquinolones also increased by 21%. It seems, therefore, that the BVA's plan has so far had no effect in improving antibiotic prescribing.

There also appears to have been a hardening of attitudes recently, with the BVA claiming that recently published research by scientists from Glasgow University suggesting that farm antibiotic was not as important a contributor to resistance in human *Salmonella* as previously thought [230], 'Calls into question policies that restrict the right of veterinary surgeons to prescribe veterinary use of antimicrobials in order to reduce resistance in humans' [231].

However, two letters published in response to the Glasgow research in the same scientific journal [233][234], criticised the way the scientists interpreted their own data and showed that their findings do not undermine the generally accepted conclusion that farm antibiotic use is the most important contributor to resistance in human *Salmonella* infections [232]. One of the letters was from the Soil Association, and the other from scientists from the United States and Denmark.

In addition to the attitudes of some veterinary surgeons, there are three major reasons driving the increasing veterinary use of these drugs: the introduction of new generic versions of the antibiotics, the advertising of the antibiotics directly to farmers and the off-label use of some of the antibiotics.

Generic versions

In recent years a significant number of generic versions of some of these antibiotics have come on the market. That has probably reduced their wholesale price, allowing vets and agricultural pharmacies to buy them more competitively. The increased number of manufacturers selling the antibiotics has almost certainly increased advertising and promotional offers to veterinary practices.

Advertising

The UK is the only member state of the EU which still permits the advertising of antibiotics directly to farmers. EU Directive 2004/28/EC required member states to ban the advertising of prescription-only medicines to 'members of the general public' [235], bringing veterinary medicines into line with human medicines. The VMD initially interpreted this to mean that advertising to farmers could no longer be

permitted, but under pressure from the pharmaceutical industry, after a period of consultation in 2005, it decided to allow advertising to continue as before, despite the Soil Association and other consumer groups expressing their support for the ban.

In 2010, the VMD opened the advertising question to consultation again, and this time the BVA also called for a ban on advertising, although a campaign was mounted by the farming press against the ban. At the end of the consultation, the VMD decided to recommend to the government that it should implement the ban, but the advice was rejected by the Farming Minister Jim Paice [236][237].

The annual increases in the use of the modern cephalosporins have been greater than the increases in the use of fluoroquinolones. This may be linked to advertising. The modern cephalosporins have been aggressively advertised to dairy and pig farmers, while the fluoroquinolones have been advertised only occasionally in the farming press.

The modern cephalosporins are also favoured by some farmers because higher levels of residues than with some alternative antibiotics are legally permitted in food, and as a result milk and meat have to be withheld from sale for shorter periods. For example the Maximum Residue Limit (MRL) of the 3rd generation cephalosporin ceftiofur permitted in retail milk is 100 parts per billion, in contrast only 4 parts per billion of amoxicillin is permitted. In the case of ceftiofur, one of the most popular injectable antibiotics on dairy farms, there is no withdrawal period for milk, which can be sold for human consumption from cows while they are receiving intramuscular antibiotics on a daily basis. One series of advertisements, no longer used but which influenced today's generation of dairy farmers after they were shown in the Farmers' Weekly about a decade ago, variously showed a dairy farmer happily asleep in bed at 7 am or still in bed and smiling, safe in the knowledge that milk from his treated cows would not be rejected at the processing dairy.

Off-label use

Antibiotics are licensed for specific purposes in specific species, but vets are permitted to extend this use to other species and other situations when, in their judgement, this is the only way to treat animals or groups of animals effectively. This is the so-called 'prescribing cascade', where vets are required to first look to antibiotics licensed in the species in question for the condition being treated, then if no suitable antibiotics are available, to antibiotics licensed in that species but for other conditions, then to antibiotics licensed only in other animals and finally to antibiotics licensed only for human use. Some antibiotics permitted in human medicine, such as chloramphenicol, are specifically prohibited in food animals.

Prescribing under the cascade, however, may only be done by way of exception, and all routine off-label use for treating groups of animals is not permitted [133]. Unfortunately, compliance with this legislation in the UK and other EU countries appears to be poor, and there is extensive evidence that vets have been prescribing antibiotics off label for routine prophylaxis [133]. Again this is an area which should be the responsibility of the VMD's Inspection and Investigations Team.

No modern cephalosporins are licensed for use in poultry, so all use is off label. Yet, government scientists in the Netherlands have reported that it is common for Dutch poultry producers to inject one-day-old chicks, or even in-ovo chicks with ceftiofur, a 3rd generation cephalosporin [238]. The European Food Safety Authority (EFSA) has pointed out that such use is not in compliance with European, or national, legislation [133]. Remarkably, this illegal off-label use seems to be the main reason why the Netherlands has such an extensive ESBL *E. coli* problem in its poultry.

Such illegal use is not restricted to the Netherlands. A VLA scientist has reported that on a British poultry farm which had both ESBL *E. coli* and ESBL *Salmonella* in its birds, ceftiofur was also injected into one-day-old chicks [238]. EFSA has reported that such use appears to be 'widespread' [133], and it seems likely that many other farms in the UK will have been doing the same, which would explain why ESBL *E. coli* are found in so many poultry companies despite no modern cephalosporins being licensed for use in poultry.

Fortunately, the British Poultry Council (BPC) has recently announced that from January 2012, British poultry producers will voluntarily cease using cephalosporins [240]. BPC Chairman John Reed said 'Cephalosporins have not been used at broiler level in the UK for five or six years. But the implication will be that parent flocks may have to deal with slightly higher mortality. Ultimately, it's a question of ensuring that antibiotics are used responsibly – the aim is to get more targeted use without compromising health and welfare'.

While this announcement is welcome, it suggests that in parent flocks, illegal off-label use of modern cephalosporins has also been common in the British poultry industry. It also shows that implementation of existing regulations has been so lax that it is possible for the industry to announce that they are now voluntarily going to comply with the law.

The BPC has also announced that the use of fluoroquinolones for disease prevention in day-old chicks will be discontinued, a further welcome development [240]. Fluoroquinolones are licensed for use in poultry, but only for treatment. So use for disease prevention is also off-label use. As such, any regular use would also have been in breach of the legislation on off-label use.

Voluntary industry announcements, while welcome, are not a sufficient response to the problem. Ultimately, the industry is not required to comply with any voluntary announcement. Producers could not be prosecuted for failing to comply, and as things stand there is nothing to prevent this decision being reversed at a later date, as happened in Quebec. Under pressure from public-health officials, poultry farmers in Quebec agreed voluntarily to suspend cephalosporin use in 2005-2006, but subsequently began using the drugs again in late 2007. The Public Health Agency of Canada was able to show that during the voluntary ban, cephalosporin resistance in human *Salmonella* fell sharply, but began rising again as the ban ended [241][242]. A Danish government scientist described the Canadian data linking antibiotic use in animals with resistance in humans as amongst the strongest he had seen and said that 'Taken in context with all the other knowledge we have, anyone still opposing a link between antibiotic use in food and animal production and its direct impact on human health does so for other reasons besides science' [242]. Despite this, poultry farmers in Quebec are still being permitted to use cephalosporins for routine prophylaxis.

Apparently illegal prophylactic off-label use is not restricted to the poultry industry. Danish scientists have reported 'systematic prophylaxis' with ceftiofur occurring on pig farms [207]. Modern cephalosporins are licensed for use in pigs, but in Denmark only for the treatment of respiratory disease. Despite this, the researchers found that on eight out of ten pig farms examined that were using modern cephalosporins, it was being injected into one-day-old piglets for routine prophylaxis.

It is possible that similar off-label use is occurring in the British pig industry, but no one has yet investigated the matter. The VLA has called for the role of off-label use of antibiotics in the selection of ESBL *E. coli* to be investigated [111].

Furthermore, according to Dr Dai Grove-White from Liverpool University, the 3rd generation cephalosporin ceftiofur 'is used routinely for almost all "infectious conditions" of dairy cattle' [243]. On large dairy farms, where the use of antibiotics is significant, it is common for vets to write out prescriptions for significant quantities of antibiotics which can then be bought either from the vet or (if cheaper) from licensed pharmacies. Once these drugs are on the farm there is usually no monitoring by the vet or anyone else to check on the reasons for their use. Farmers are required to record each time they use antibiotics, but inspections of their records by Trading Standards officers do no more than ensure that records are being kept. Veterinary surgeons can also ask to inspect the records and have the ability to scrutinize them intelligently. However, since many vets earn a substantial part of their income from the sales of antibiotics, their scrutiny is hardly independent. As such, farmers often get away with using these antibiotics for a wider range of conditions than they should, not because other antibiotics would not be effective, but because they do not need to worry about withdrawal periods.

9.3. Livestock species with higher antibiotic usage have more resistance

Scientists from Bristol University and the VLA compared resistance in *E. coli* from pigs, with that from cattle and from sheep. As mentioned in section 9.1., pigs account for approximately 60% of farm antibiotic use in the UK, cattle for just 4% and sheep for less than 0.5%. These differing usage levels were reflected in the levels of antibiotic usage found in each species: the scientists tested *E. coli* from healthy animals at slaughter, and found that 3% of 836 isolates from sheep, 5.7% of 836 isolates from cattle and 92.1% of 2480 isolates from pigs were resistant to at least one antibiotic.

The scientists said that the resistance was of concern because 'it can potentially spread to humans, either via direct colonization of the human gut by animal strains of *E. coli* or through transmission of resistance genes to resident bacteria in the human gut' [244].

9.4. Organic antibiotic restrictions lead to lower resistance levels

The use of all antibiotics in organic farming is restricted under national and EU legislation to the treatment of ill animals when effective alternatives are not available. Using antibiotics in pig and poultry feed and water for routine prophylaxis, as frequently occurs in conventional farming, is not permitted.

The Soil Association also placed additional limitations on the use of fluoroquinolones, in 2004, and modern cephalosporins, in January 2009. These restrictions banned their use except in extreme situations where it could be shown that no other antibiotic was likely to be effective in saving an animal's life, and even then use is only permitted for treating individual animals [245]. The restriction on use in individual animals means that these antibiotics can effectively not be used in Soil Association certified poultry production.

In non-organic farming, dairying uses the most antibiotics, after the pig and poultry industries. Beta-lactam antibiotics, in particular, are widely used in dairy cows for the treatment and prevention of mastitis. Dry-cow therapy is routinely used in non-organic dairy herds after the final milking of a lactation. A beta-lactam antibiotic, such as ampicillin, cephalonium, cloxacillin or cefquinome (a 4th generation cephalosporin), is infused into each teat. This is done in an attempt to provide protection against summer mastitis, which can occur during the period when the cow is not giving milk, and to reduce the chances of mastitis at the beginning of the next lactation.

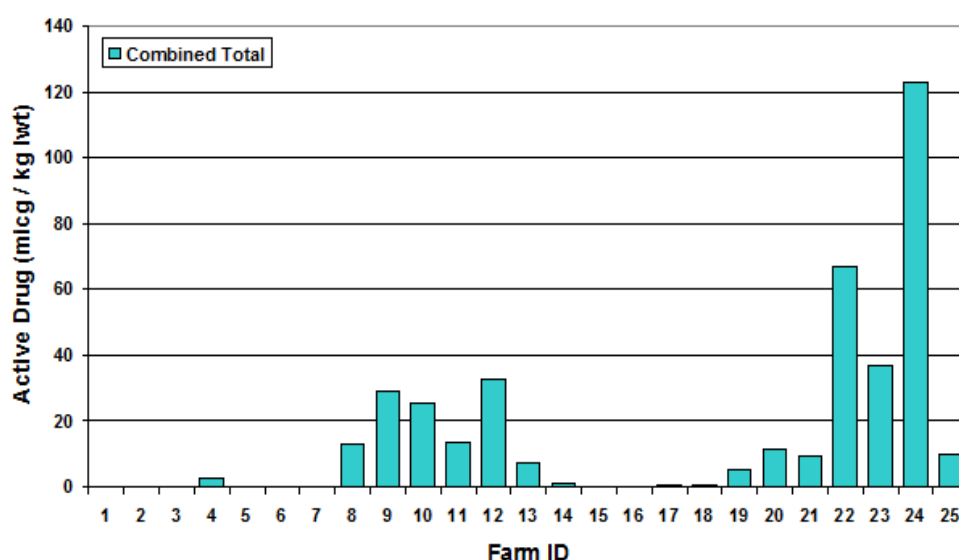
In organic farming, on the other hand, dry-cow therapy is strongly discouraged, although special permission is given under certain circumstances for individual cows with a history of recurrent mastitis.

The high levels of use of beta-lactams, including modern cephalosporins, on non-organic farms for treating and preventing mastitis, is clearly a major contributor to the ESBL *E. coli* problem on British dairy farms. Scientists have often assumed that intramammary infusions do not contribute to the resistance problem in humans, because these treatments would only minimally affect bacteria in the animal's gut, and furthermore any resistant bacteria in milk will be killed during pasteurisation. However, on most dairy farms, waste milk produced during the withdrawal period of the antibiotic, which cannot be sold for human consumption, is fed to calves on the farm. A VLA survey found that this practice occurs on 81% of British dairy farms surveyed [246]. Furthermore, it has been shown that calves fed such waste milk develop higher levels of antibiotic-resistant bacteria in their gut than those that are not fed the milk, and that the resistant bacteria persisted after feeding of the waste milk had ceased [247].

We have not been able to find any data comparing antibiotic use and resistance in *E. coli* from cattle on organic and non-organic farms in the UK. However, for pig and poultry farms, data from publicly funded research shows that levels of antibiotic use and antibiotic resistance in *E. coli* are much lower on organic farms. VLA research, published in 2006, compared antibiotic use on seven organic poultry farms and five organic pig farms with six non-organic poultry farms and seven non-organic pig farms. They found much lower levels of antibiotic use on the organic farms: whereas all the non-organic farms used antibiotics, six of the seven organic poultry farms and two of the five organic pig farms did not use antibiotics at all during the entire two-year study [188].

Per kilogramme of meat produced, the non-organic pig farms used between 13 times and 330 times more antibiotics than the highest-consuming organic pig farm. All of the antibiotics used on the organic farms were injected – there was no use in feed or water. The only organic poultry farm that had used antibiotics during the two-year period had used them on a single occasion due to an outbreak of yolk-sac infections in incoming chicks. Graph 6 compares the antibiotic use on the 25 farms [188].

Graph 6. Use of antibiotics (microgramme of active ingredient per kg of meat produced) on organic poultry (1 to 7) and pig (14-18) farms compared with non-organic poultry (8-13) and pig (19-25) farms (source: [188])



We do not have precise data on the average number of treatments of non-organic chicken in the UK, but in the Netherlands non-organic chickens get on average four courses of antibiotics in their short 42-day

lives [127]. The VLA study found that two of the non-organic poultry farms were routinely dosing all chicks for the first three days of after their arrival on the farm with the antibiotic combination lincomycin-spectinomycin, in order to prevent yolk-sac infection. The VLA said 'It is over the course of these 3-days that this change in multiple-resistance occurs in the faecal *E. coli* population. Thus it would seem that the use of this broad-spectrum antimicrobial could be selecting for the survival of multiple-resistant *E. coli*. Furthermore this level of multiple-resistance remains high throughout the growing cycle'.

The VLA also said 'Such prophylactic use of lincomycin-spectinomycin is now very common in the UK broiler industry' [188]. An industry source has told the Soil Association that almost all farm-assured non-organic chickens are still being put on routine prophylactic antibiotics of lincomycin-spectinomycin the day they are hatched [248].

In the VLA study, the much lower levels of antibiotic use on the organic farms were reflected in the lower levels of resistance. The *E. coli* taken from the poultry farms were tested for their resistance to ten different antibiotics. The median number of antibiotics to which the *E. coli* from the organic poultry farms were resistant was just one, whereas for the non-organic poultry farms it was five. Fluoroquinolone-resistant *E. coli* was found on four of the conventional pig farms in 9 to 66% of pooled faecal samples, but in just a single sample from one of the organic pig farms. The levels of ampicillin and gentamicin resistance in the pig samples were found to be correlated with total antibiotic use, which was much higher on the non-organic farms than on the organic farms [188].

Research funded by the Scottish Executive, and published in 2000, also found much lower levels of resistance in organic pigs than in non-organic pigs. On intensive farms, it was found that resistance in *E. coli* was 'widespread', even when the pigs received minimal antibiotics in their feed. Resistance to tetracycline was particularly, at 'up to 100% in pigs prior to slaughter'. In contrast, they found that on small organic pig farms there were much lower levels of carriage of resistant *E. coli* (0-10%) [249].

Despite these extremely low levels of antibiotic use and resistance found in these studies on a number of British organic farms, a recent study in the Netherlands has found widespread contamination of retail organic chicken with ESBL bacteria: 84% of organic samples had ESBL bacteria compared with 100% of conventional samples. The average numbers of ESBL bacteria on the organic samples was significantly smaller on the organic meat than on the conventional meat (four times lower), but these are nevertheless surprisingly high contamination rates of organic produce. As the authors suggested, there are several likely explanations for this. One is that organic poultry producers in the Netherlands are buying in one-day-old chicks which are already contaminated with ESBL *E. coli*. It has been shown in the Netherlands that ESBL bacteria are found at all levels of the poultry production chain. This is partly due to the routine prophylaxis of one-day-old chicks with modern cephalosporins. The scientists also suggested that contamination of organic chickens with ESBL bacteria from non-organic chickens could occur at the abattoir, or at retail level. The scientists also reported that the ESBL bacteria from the organic meat had much lower levels of resistance to tetracycline, a widely used antibiotic on non-organic poultry farms. They said that this indicated that there was lower level of antibiotic use on organic farms [127].

According to Soil Association organic standards, producers may only buy in non-organic livestock if organic livestock, or in conversion livestock, are not available, and then only with the Soil Association's approval. In 2011 approximately 80% of organic chickens certified by the Soil Association came from organic hatcheries, and the remaining 20% came from a non-organic hatchery [250].

10. The dangers of low concentrations of antibiotics in feed

Recent research has shown that at remarkably low concentrations, antibiotics have the ability to favour the growth of resistant *E. coli* and *Salmonella* over sensitive *E. coli* and *Salmonella*. Regarding *E. coli*, Swedish scientists have shown that at concentrations of just 100 picograms per ml of the fluoroquinolone antibiotic, ciprofloxacin, resistant bacteria would outgrow sensitive bacteria [251]. This equates to just 0.1 part per billion (ppb) and is well below the traditional 'Minimum Inhibitory Concentration' (MIC), the lowest concentration at which the antibiotic visibly inhibits sensitive bacteria.

It is also well below the Maximum Residue Limit (MRL) of 100 ppb for permitted residues in milk and meat of enrofloxacin, a fluoroquinolone closely related to ciprofloxacin. This suggests that residues below the MRL of fluoroquinolones in food could be selecting for resistant *E. coli* in the human gut. The authors also suggested that the release of antibiotics into the environment, in human sewage or animal manure, could also be at high enough concentrations to select for resistant bacteria and could explain the environmental spread of resistance.

The Swedish research findings were similar to earlier research carried out by British scientists who found that extremely low levels of tetracycline antibiotics could affect the growth of sensitive *E. coli* taken from the faeces of pigs and a calf [252]. They showed that concentrations of just 0.25 nanogrammes per ml, which equates to 0.25 ppb, had an effect on bacterial growth. This is also well below the MRL of 100 ppb for tetracycline in meat and milk.

The Swedish scientists also warned of the particular dangers of low levels of antibiotic use: some resistance mutations can have a 'fitness' cost, as a result of which the resistant bacteria with the mutation do not grow as well as sensitive bacteria in the absence of the antibiotic. In this case, when the antibiotic is removed, the sensitive bacteria tend to grow back, and levels of resistance fall. Other types of resistance, on the other hand, appear to have little fitness cost. At high levels of antibiotic use, bacteria are selected according to whether or not they are resistant, and the fitness cost of a resistance mutation is not relevant. But at low concentrations, below the MIC, sensitive bacteria are still growing, but at a slower pace, and can still outcompete bacteria with the resistance mutation with the high fitness cost. The favoured bacteria at low concentrations, however, are those that have a mutation with low fitness costs. If low antibiotic concentrations are maintained for a sufficient length of time, the 'fit' resistant bacteria will come to dominate, and when the antibiotic is removed, the sensitive bacteria will not be able to grow back as easily. High levels of antibiotic resistance will remain, despite the antibiotic no longer being used.

The Swedish scientists only tested the growth of resistant and sensitive *E. coli* in different low concentrations of fluoroquinolones, and they carried out similar experiments for *Salmonella* in the presence of tetracyclines. So we cannot draw firm conclusions for all antibiotics, but it seems likely that many antibiotics will have similar effects at very low concentrations.

This has serious consequences for the large-scale farm use of low concentrations of antibiotics in animal feed and water: when farm antibiotics are used for disease prevention, rather than for treating an actual illness, the concentrations used are generally much lower than the therapeutic doses [253]. These low doses are likely to be not only selecting for resistant bacteria, but bacteria with resistance mutations with low fitness costs. Furthermore, the widespread dissemination into the environment of low concentrations of antibiotics in manure, and low levels of residues in food may be compounding the problem.

11. References

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