UK publicly funded research on microbial antibiotic resistance in relation to the safety of food

Report from the Microbiological Safety of Food Funders Group

March 2007

UK publicly funded research on microbial antibiotic resistance in relation to the safety of food
Research from 1990 to 2006

OVERVIEW

This report is the output of a review by the Microbiological Safety of Food Funders Group (MSFFG) of the research relating to microbial antibiotic resistance in relation to the safety of food supported by its member organisations from 1990 to the end of 2006.

Although the primary driver for the emergence of antibiotic resistant bacteria in humans is believed to be the use, or overuse, of antibiotics in human medicine, the Report on Microbial Antibiotic Resistance in relation to food safety (ACMSF (1999)) highlighted the need to better understand the role of the veterinary use of antibiotics and the effect of this on bacteria in the food chain. The area of the relationship between the use of antibiotics, the emergence of antibiotic resistance and interactions of these with the food chain is very complex. Research addressing these combined issues is difficult to design and execute so as to be able to draw clear conclusions whilst avoiding confounding issues. Nevertheless, MSFFG member organisations have supported a body of research in this area. The overall observation is that there is evidence that the use of antibiotics in animals, whether therapeutically, prophylactically or as growth promoters, leads to a risk of the transfer of antibiotic resistant bacterial populations from farm animals to humans through the food-supply chain to the consumer, thus potentially posing a risk to human health.

Much of the relevant research has focused on antibiotic resistance and the mechanisms involved in this in *Salmonella* and *Campylobacter*, the two most common causes of bacterial food-poisoning in the UK. In particular, the development and management of resistance to quinolone antibiotics has been studied, with common findings of a rapid development of antibiotic resistance, which is likely to bring with it multiple antibiotic resistance. The research also points to the development of resistance to non-antibiotic antibacterial agents such as disinfectants.

With concerns over the development of antibiotic resistance, this is an important area for research, including in relation to the microbiological safety of food. There are several relevant areas where there may be a need for additional research, in particular understanding of the epidemiology of antibiotic resistance as this relates to food-borne illness in humans and the possible transfer of antibiotic resistant bacterial populations from farm animals to humans. Other areas where further research may be required include the occurrence and spread of extended spectrum β lactamases in a variety of bacterial species. There is some ongoing research into alternatives to antibiotics for treating animal and human infections that may benefit from expansion.

LAY OVERVIEW

Antibiotic resistance is the term used to describe the ability of a bacterium to resist the effects of antibiotics and therefore to survive and multiply in their presence. Antibiotics are substances used to treat infectious illnesses in humans and animals. Certain antibiotics have in the past been used, at low concentrations, as growth promoters in farm animals, leading to enhanced weight gain and productivity: such usage has been now been prohibited within the EU.

As a result of the use of antibiotics in human and animal health, there has been a significant development of antibiotic resistance in bacteria which cause illness in humans and, separately, in animals. Whilst the great majority of the resistance seen in bacteria that cause human infections is thought to be due to the use of antibiotics in human medicine, the concern of research in this report is whether there is a link between the occurrence of antibiotic resistant bacteria in farm animals and the occurrence of antibiotic resistant bacteria in humans by transfer of such resistance through the food supply chain.

This is a complex area of research, and whilst it is established that the use of antibiotics leads to the development of antibiotic resistant bacteria, there is a relative lack of data demonstrating transmission of antibiotic resistant bacteria from farm animals to humans through the food chain. The evidence suggests that there may be a link, but that it is probably only a minor contributor to the problem of the occurrence of antibiotic resistant bacteria in humans, which is generally unrelated to the food-supply chain.

Table of Contents

UK publicly funded research on microbial antibiotic resistance in relation to the safety of food

Research from 1990 to 2006

1. INTRODUCTION

1.1 The Microbiological Safety of Food Funders Group (MSFFG) has previously published reports giving an overview of the research funded by member organisations of the MSFFG relating to various food-borne pathogens including Verocytotoxin-producing *Escherichia coli*[1], *Campylobacter*[2], *Salmonella*[3], *Listeria monocytogenes*[4], *Yersinia enterocolitica*[5], food-borne viruses[6], *Mycobacterium avium* subsp. *paratuberculosis*[7], *Brucella*[8], Farm and Abattoir Waste[9] and *Clostridium*[10]. As part of the ongoing process of considering research into all food-borne pathogens supported by MSFFG members, the group has undertaken a review of research on microbial antibiotic resistance as it is relevant to food-borne disease, as recorded in this report.

1.2 This report gives an overview of research, undertaken in the UK and funded by member organisations of the MSFFG, related to the occurrence and significance of antibiotic resistance in bacteria as it relates to and affects the microbiological safety of food. Research initiated during the period from 1990 to the end of 2006 is summarised and set in the context of other research and issues within the UK and overseas. In addition an assessment is made of those areas where further research may be needed.

---

[1] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/vtec

[2] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/campylobacter

[3] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/55669

[4] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/listeria

[5] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/yersinia

[6] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/msffgfbv2005

[7] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffgmapreport

[8] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/mffsgbrucellareport

[9] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/mffsgfarmabatoirreport

[10] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/clostidium

1.3 Strictly speaking, antibiotics are defined as substances, produced by or derived from bacteria, which inhibit the growth of other bacteria. Strictly they do not include chemically synthesised compounds such as sulphonamides and quinolones. However, common usage generally extends to include such agents, and this approach is taken in this report (as it is in the ACMSF Report on Microbial Antibiotic Resistance (1999)).

1.4 The development by a bacterium of resistance to one or more antibiotics can occur either by acquisition of the relevant genetic material conferring resistance from a source outside of the bacterium, or by the mutation of one or more genes within the bacterial genome. In the latter situation, the mutation may either be in the antibiotic target, thus rendering the bacterium less susceptible to the antibiotic, or in the genes of a protective mechanism, leading to an increase in the effectiveness of this. Acquisition of DNA from outside the bacterium can occur through several mechanisms, most commonly through conjugation (for a review, see ACMSF Report on Microbial Antibiotic Resistance (1999)). Conjugation is also a significant mechanism for transmission of multiple antibiotic resistances between bacteria. With all the mechanisms, the evidence is that the use of antibiotics encourages the development and spread of antibiotic resistant bacterial strains that, by virtue of the protective genes they have acquired or developed, can grow and multiply despite the presence of antibiotics.

1.5 When considering the development of antibiotic resistance in bacterial populations of farm animals, an added feature is the use that has been made of antibiotic growth promoters. Their utilisation has recently been considerably restricted as a result of EU legislation[11], partly because of concerns that excessive use was leading to an increase in occurrence of antibiotic resistant bacteria in farm animals. The concern was that this represented a risk to animal and human health through the reduction of usefulness of certain antibiotics as therapeutics.

1.6 Antibiotic resistance is a problem in both animal and human health. The occurrence of antibiotic resistant bacteria reduces the effectiveness of antibiotics themselves, thus limiting the options for management of infectious diseases. This in itself is a problem, but it is made more serious if the genes for antibiotic resistance can be transferred to bacterial populations which were previously sensitive. It is accepted that this occurs within human bacterial populations and, separately, farm animals, where the occurrence of antibiotic resistance in the past has been exacerbated by the use of antibiotic growth promoters (Khachatourians GC, (1998)). In relation to the microbiological safety of food, the question arises as to whether there is transfer of antibiotic resistance from bacterial populations in farm animals to those of humans. This can be either through the transmission of the antibiotic resistance between bacterial populations

[11] http://www.vmd.gov.uk/General/Antibiotic/poag.htm

or, more likely, the infection of humans with antibiotic resistant bacteria from farm animals. Either of these routes to transmission of antibiotic resistance to humans presents an additional risk to human health and to the use of antibiotics in the clinical context.

1.7 It was acknowledged by the Advisory Committee on the Microbiological Safety of Food (ACMSF) (Report on Microbial Antibiotic Resistance in Relation to Food Safety (1999)) that there have only been a few studies on the emergence of antibiotic resistant bacteria in farm animals, and that there is little research on the transmission of antibiotic resistant bacteria from farm animals to humans. However, after assessing the scientific evidence, this group concluded, as have other organisations, that this is a route by which antibiotic resistant bacteria capable of causing disease may be introduced to humans. The research supported by the member organisations of the MSFFG and recorded in this report addresses aspects of this issue.

1.8 Aquaculture and horticulture are also routes by which antibiotic resistant bacteria may be introduced into the human food chain. The ACMSF recognised the former as of relevance to the UK in making two recommendations relating the use of antibiotics in aquaculture in the Report on Microbial Antibiotic Resistance in Relation to Food Safety (1999). No research in either area has been supported by MSFFG member organisations and the topics are excluded from consideration in this report.

2. METHODS

2.1 This report is based on those research projects that are funded by the member organisations of the MSFFG. At the time of writing this report, these were the Food Standards Agency (FSA), the Department for Environment, Food and Rural Affairs (Defra), the Biotechnology and Biological Sciences Research Council (BBSRC), the Department of Health (DH), the Department of Agriculture and Rural Development, Northern Ireland (DARD), the Environment Agency, the Food Safety Promotion Board (FSPB), FSA Scotland, FSA Wales, FSA Northern Ireland, the Health Protection Agency (HPA), the Meat and Livestock Commission (MLC), the Medical Research Council (MRC), the Scottish Executive Environment and Rural Affairs Department Science and Research Group (SEERAD SRG) and the Scottish Executive Department of Health (SEDH).

2.2 The MSFFG project database[12] was used to identify projects for inclusion in this report. The database was searched using a variety of

[12] The MSFFG maintains a database containing information about research projects in the area of the microbiological safety of food that are funded by the members of the MSFFG. Members of the Group provide the project information from their respective project record systems. The earliest projects within the database were initiated in 1990. Some historic project data from member organisations joining the MSFFG in 2005 (eg the EA, HPA, MLC, MRC) may be unavailable to the database. www.msffg.org.uk

terms relating to antibiotic resistance, including 'antibiotic resistance', 'antimicrobial', 'antimicrobial resistance' and 'quinolone(s)'. In addition, members of the MSFFG were requested to identify any projects which might have been omitted from the MSFFG project database. This gave a total of 48 projects which are listed in Appendix 2. The earliest of these projects was initiated in 1995, although the MSFFG database includes research reports dating from 1990. Three quarters of the projects have been initiated since the start of 2000.

2.3 Studentships have been omitted from consideration.

2.4 Research funded by other agencies, including the Wellcome Trust, Royal Society and NHS Scotland as well as international research is not included within the body of the report. However, a summary of research funded through these bodies is given in section 3 below.

3. Research supported by other funding bodies

3.1 Internationally, much of the research and surveillance relating to the transfer of antibiotic resistant bacteria from farm animals to human food through the food chain is embedded in research projects that address other issues, such as studies of the development of antibiotic resistant bacteria in food animals. In this context, the United States Department of Agriculture, the US National Institutes of Health and other US bodies, as well as the European Union through the Framework Programmes, do fund indirect research and surveillance studies that provide some insight into the transmission of antibiotic resistant *Salmonella* and other bacteria from farm animals to humans. In addition, the European Union Sixth Framework Programme includes several research projects examining alternative animal husbandry approaches in the light of the anticipated reduction in antimicrobial growth promoters.

3.2 The recent 8th Congress of Chemotherapy and Infection focused on antimicrobial resistance showed the same pattern, with the principal themes and presentations considering antibiotic resistance from a healthcare perspective. An emerging theme was the importance of extended spectrum β lactamase (ESBL) producing *Enterobacteriaceae*. A conference report is given at Appendix 2.

4. RESEARCH SUPPORTED BY THE MSFFG

4.1 Background concerns

The frequency of occurrence of antibiotic resistant bacteria in humans and farm animals is known to be increasing, and is a cause for concern (ACMSF (1999)). Research funded by the member organisations of the MSFFG has supported this view. In 1995 it was found that 13% of faecal *Campylobacter* isolates showed resistance to quinolone

antibiotics (**DH 226**). Investigation of isolates from the Intestinal Infectious Disease study in 2000 found that whilst the majority of *Campylobacter jejuni* isolates were sensitive to ciprofloxacin and erythromycin, 26% of isolates showed resistance to ciprofloxacin (**FSA B10002**). A study in Ireland published in 2004 found that of *Campylobacter* isolated from clinical and food samples, 81% of food isolates and 93% of clinical isolates were resistant to at least one antibiotic (**Food Safety Promotion Board 00-RESR086**).

4.2 Molecular Biology

4.2.1 The transmissible or inheritable component of antibiotic resistance is encoded in DNA and is a part of the genotype of the bacterium. The mutated or introduced genes are not, in themselves, the mechanism of antibiotic resistance. Their expression as proteins with specific functions leads to the phenotype of antibiotic resistance. Molecular biology studies antibiotic resistance both at the level of DNA and the expressed proteins. The former provides opportunity to understand how resistance may arise, and be transmitted, as well as identifying specific resistance factors. Study of the proteins and pathways involved in antibiotic resistance can also assist in identifying specific factors but is particularly important in understanding the mechanisms by which bacteria resist the effect of antibiotics.

Detection and identification

4.2.2 Modern molecular biology techniques are being applied to developing rapid and accurate tests for detecting the presence of bacteria which have developed or acquired antibiotic resistance. Several different analytical techniques are being developed for the identification of specific resistance genes of importance to human and animal health in *Salmonella enterica,* including DNA microarrays, (**Defra VM02136**) and Variable Number Tandem Repeats (VNTR) (**Defra VM02205**). Molecular subtyping and fingerprinting, such as rRNA LightCycler assays and pulse field gel electrophoresis (PFGE) have been used to identify genomic markers of antibiotic resistance in *Campylobacter*, *Salmonella* and *Enterococcus.* (**Defra VM02105**). LightCycler technology has been used to develop assays for the rapid identification of mutations in the gyrA gene for use in studies examining fluoroquinolone resistance (**FSA B10001**).

4.2.3 Microarrays to identify pathogens and monitor the movement of genes, including antibiotic resistance genes, are being developed (**MRC G0300020**). It is anticipated that these will be applicable to human and animal bacterial populations.

4.2.4 Other research seeks to identify changes in cell envelope protein profiles of *S. enterica* serovar Typhimurium with multiple antibiotic resistance (MAR) gained through the acquisition or up-regulation of efflux pumps that could then be used to develop a test to identify

isolates which had the MAR phenotype (**Defra OD2011**).

4.2.5 Standardised procedures are also being developed for the identification and differentiation of methicillin-resistant *Staphylococcus aureus* (MRSA) of human and animal origin, so as to inform understanding of the origin of such isolates (**Defra OD2020**).

Mechanisms of antibiotic resistance

4.2.6 Several different mechanisms of antibiotic resistance have been the subject of research relevant to the microbiological safety of food. Efflux is known to be a major mechanism for MAR in Gram negative and other bacteria (Levy (1992)) and mutation(s) in the gyrA gene provide a mechanism for the development of resistance of Gram negative bacteria to fluoroquinolones (Everett and Piddock (1998)). Research with *Campylobacter* and *Salmonella* confirmed that both these mechanisms were involved in the resistance to fluoroquinolones (**Defra OZ0132, VM02100**). Further research had been initiated investigating the mechanisms for multiple antibiotic resistance in *S. enterica*, where there is some evidence that efflux is not the only mechanism (**MRC G0501415**).

4.2.7 Repression of gene expression through gene silencing has been identified as a mechanism for loss of antibiotic resistance phenotype in *Escherichia coli* (**Defra OD2007**). This novel mechanism is to be further investigated, in order to determine details of the mechanism in *E. coli* and whether such a mechanism occurs in other species (**Defra OD2022**).

Evolution of antibiotic resistance

4.2.8 Evolution, or development by mutation, of antibiotic resistance, leads to the growth of bacteria with mutations that can give improved growth and survival profiles in the presence of antibiotics or other antimicrobial agents. Understanding such evolution is important in the context of the use of preservatives in the food industry and antibiotics in animal husbandry. Studies of the mechanisms of such evolution have been undertaken with a view to increased understanding of how environmental pressures exert their effect (**BBSRC 4311218**).

Transfer of antibiotic resistance

4.2.9 The study of the transfer of antibiotic resistance elements between bacteria provides benefits from insight at a molecular level. Studies include modelling of the competitive interactions between resistant and sensitive strains (**BBSRC 772**), work with food-borne human pathogens and food-use industrial strains (**BBSRC 4311218**, **BBSRC D15925**). In the last of these projects, the history of tetracycline resistance transfer was examined with a view to understanding the impact of commensal bacteria in the gut harbouring antibiotic resistance factors which can be transferred to pathogens.

4.3 Antibiotic resistant bacteria in farm animals

4.3.1 In relation to the microbiological safety of food, farm animals represent a major source of bacteria expressing antibiotic resistance which could be introduced into the food-supply chain. In particular, there is evidence that bacteria resistant to antibiotics have arisen in food animals as a consequence of the use of such agents in farming and animal husbandry (ACMSF (1999)). Research supported by MSFFG member organisations in the last fifteen years has sought to develop an understanding of the nature and causes of this issue.

The occurrence of antibiotic resistance in farm animals

4.3.2 Most of the projects covered in this report focus on *Campylobacter* and *Salmonella,* which are known to develop antibiotic resistance and present a possible risk to human health through food. There are also a number of projects specifically examining the occurrence of other species of antibiotic resistant bacteria in farm animals. These include *Enterobacteriaceae* in farmed cattle and sheep (**SEERAD SAC/147/97**), MRSA (**Defra OD2020**) (also investigated in companion animals, **Defra OD2019**) and *Escherichia coli* (**Defra OD2002**). Research in this last project found that *E. coli* expressing multiple antibiotic resistance occurred in 19 out of 21 sheep and cattle grazing farms investigated.

The impact of antibiotics as growth promoters on antibiotic resistance

4.3.3 Antibiotics were formerly used as growth promoters for farm animals. Research projects have considered the impact of this practice on the development of antibiotic resistance in bacteria in poultry (**Defra OD2001**) and pigs (**SEERAD SAC/137/97**).

4.3.4 More recently, research has focused on the impact of their withdrawal on the persistence or otherwise of antibiotic resistant microbial populations. However, initial research cast doubt on the value of extensive field studies in monitoring the decline of antimicrobial resistance and the results from of an alternative modelling system that was subsequently developed did not lead to clear conclusions (**Defra VM0292**). Research elsewhere has demonstrated that for a number of antibiotic growth promoters, their withdrawal leads to a marked reduction in the occurrence of antibiotic resistant bacteria in farm

animals (Aarestrup *et al.* (2001)).

The impact of therapeutic antibiotics on antibiotic resistance

4.3.5 The therapeutic use of antibiotics was shown to lead to a significant and rapid increase in the presence of antibiotic resistant bacteria in the gut of pigs (**Defra OD2003**). It was also observed that low level multiple antibiotic resistance readily developed in both *Campylobacter* and *Salmonella* when exposed to antibiotics and/or disinfectants by mutation in the genes involved in the efflux systems (**Defra OD2004**).

4.3.6 Treatment of poultry with fluoroquinolones led to an increase in the numbers of ciprofloxacin-resistant *Campylobacter* present in the flock, due to selection of new mutations and previously resistant bacterial strains (**Defra OZ0501**, **VM02100**). However, it was also noted in both projects that fluoroquinolone resistant *Campylobacter* could be isolated from poultry in the absence of any recent antibiotic treatment. This contrasted with the occurrence of fluoroquinolone resistant *Campylobacter* in pigs, which was very rare in the absence of treatment (**Defra VM02101**). Research is now in place to determine whether the use of antibiotics affects the population dynamics of *Campylobacter* spp in general, including general patterns of antibiotic resistance (Defra **VM02200**).

4.3.7 Resistance to the macrolide antibiotic erythromycin in campylobacters in pigs is presumed to be related to the therapeutic use of tylosin. In research to determine whether there was a similar link in poultry, it was found that use of Tylan soluble®, a commercial form of tylosin, could eradicate both *Campylobacter* and enterococci from colonised chickens without leading to the development of erythromycin-resistant bacteria **Defra OZ0502**.

4.3.8 The effect of repeated use of antibiotics on the acquisition and persistence of antibiotic resistant bacteria in pigs is being investigated (**Defra OD2015**). This work is being followed with research to determine whether different antibiotic dosing regimes can prevent the development of fluoroquinolone resistant *Salmonella* and *E. coli* (**Defra VM02201**). In addition, research has been initiated to identify novel antibiotic resistance genes developing in the bacterial populations of pigs exposed to different antibiotic regimes (**Defra OD2014**).

4.3.9 It was found in pigs that the withdrawal of antibiotics led to a reduction in the number of bacteria carrying antibiotic resistance genes (**Defra OD2003**). Similarly, in poultry fluoroquinolone resistant strains of *Campylobacter* persisted for up to four weeks after treatment, and then disappeared (**Defra OZ0501**). Further research using a pig model is examining the impact on the withdrawal of antibiotics on the persistence of antibiotic resistant strains of *E. coli* and *Enterococcus* (**Defra OD2007**). Bacterial isolates which demonstrate an apparent loss of antibiotic resistance will be investigated in order to determine

the mechanism of this loss.

Environmental sources of antimicrobial resistance genes

4.3.10 Transfer of antibiotic resistance factors may also occur in the environment, as well as in direct association with farm animals. Concern that this was the case led to research on the transfer of resistance between microbes in stored and spread farm wastes (**Defra OD2002, OD2005, OD2008**). Transfer of resistance factors between bacteria was observed in model field experiments in June, but not in March, suggesting that antibiotic resistance factors could be transferred to non-resistant strains under certain circumstances (primarily increased temperature) (**Defra OD2008**). It was observed that when manures carrying multiple-resistant *E. coli* were spread on pasture, there was extensive occurrence of these strains in the environment. Calves and sheep were also found to have acquired strains exhibiting multiple resistance, but only on one occasion was there any evidence that the source was farm manure (**Defra OD2002**). However, no transfer of antibiotic resistance factors was observed in laboratory studies and models of store biological waste (**Defra OD2008**) nor in laboratory or field studies when manures spiked with tetracycline resistant *E. coli* were spread (**Defra OD2005**). It was concluded that transferable antibiotic resistance existed in slurries but that the actual transfer of antibiotic resistance did not occur to a measurable level in the environment of stored and spread manure (**Defra OD2005**). The overall conclusion of these studies was that there is unlikely to be a measurable risk of antibiotic resistance factors spreading through the use of biological farm waste

4.3.11 Wild animals offer a possible source of antibiotic resistance for farm animals. Studies have been undertaken to determine the occurrence and persistence of antibiotic resistance in wild rodent populations, including investigation of how antibiotic resistance could persist in the absence of antibiotic therapy (**Defra OD2009**). Antibiotic resistance was found in faecal *E. coli* of most of the rodent populations studied, although the frequency of occurrence was variable. There was some evidence that levels of antibiotic resistance were higher when there was direct contact between rodents and livestock, and it was found that the antibiotic resistant *E. coli* isolated from chickens, house mice and other wild rodents from one site showed the same resistance profiles. The same project noted that there were antibiotic resistant *E. coli* in soil and water samples taken for one component of the study.

4.3.12 Non-antibiotic antimicrobial agents have also been found to promote the development of antibiotic resistance (**Defra OD2010**, see section 4.6.6 below).

4.4 The relationship between antibiotic resistant bacteria in the food supply chain and humans

4.4.1 The relationship between antibiotic resistance in bacteria found in farm animals and humans is clearly important. Correlation between the two populations of antibiotic resistant bacteria two would suggest that there is transfer of elements of the bacterial population and, possibly, resistance genes, between farm animals and humans. This presents both challenges and opportunities in the management and understanding of antibiotic resistance.

4.4.2 A number of research projects have studied the commonality of antibiotic resistance between different species and man. Strains of *S.* Typhimurium exhibiting similar patterns of multiple antibiotic resistance were isolated from both farm animals and humans and it was concluded that there is no difference in the identity of human and food animal isolates (**FSA B10001**). An earlier project found that isolates of *S.* Typhimurium DT104 from cattle and humans with the same MAR phenotype contained the same inserted genes, suggesting that one clone of the bacterium was spreading across both species (**DH 240**). Other research examined the importance of the antibiotic resistance status of bacterial populations in poultry and pigs in the development of fluoroquinolone resistant *Campylobacter* in humans (**FSA B10004**). The relatedness of antibiotic resistant strains of *Campylobacter* and *E. coli* isolated from pigs and poultry and those infecting humans has been considered (**Defra VM02101**) as has the possible emergence of antibiotic resistance in *E. coli* O157 (**FSA B08003**). The possibility that novel mechanisms of tetracycline resistance may develop in porcine bacterial populations, and then be transferred to humans to the detriment of the use of new tetracycline antibiotics is also being explored (**Defra OD2014**).

4.4.3 Several recent projects are examining the transfer of antibiotic resistance from animal bacterial populations to clinical strains. One project aims to determine the relationship between animal commensal bacteria and human pathogens, and also whether there is transmission of genetic elements encoding resistance factors from animal to clinical strains of bacteria (**Defra OD2012**). A further project investigates whether the veterinary use of glycopeptide antibiotics (eg avoparcin) could lead to the development of vancomycin resistance in *Staphylococcus aureus* in humans, as has been presumed (**Defra OD2013**).

4.4.4 It is not known whether MRSA is actually present in food-producing animals in the UK, and research has been initiated to investigate the MRSA status of cattle in England and Wales (**Defra OD2020**).

4.4.5 A national archive facility for antibiotic resistant bacteria from food has been established (**FSA B10003**). This provides a reference resource for antibiotic-resistant bacteria isolated during the course of FSA-sponsored surveys of food.

4.5 Risk and risk management

4.5.1 The occurrence of antibiotic resistant bacteria in the food supply chain represents a risk to humans through the microbiological safety of food. Assessment of the prevalence of antibiotic resistant *Campylobacter coli* and *C. jejuni* in pigs at slaughter found that the majority of isolates were resistant to tetracyclines (79% and 70% respectively) and that 85% of *C. coli* were resistant to erythromycin (**Defra VM02110**). The prevalence of resistance was higher than had been seen with previous studies of other farm animals.

4.5.2 The widespread use of antibiotics in farming has increased the selective pressure applied to the bacterial population to develop or acquire antibiotic resistance. In a survey it was found that 35 out of 40 pig farmers used antibiotics or growth promoters in at least one daily feed (**Defra VM02103**) although in a larger survey of pig producers, 61% indicated that the use of growth promoters was not justifiable. A different study found that 22% of pig units did use antimicrobial growth promoters, and around 30% of poultry production also utilised such growth promoters (**Defra VM0294**). Their use in beef and dairy cattle management and with sheep was found to be negligible. Both these surveys were taken after the initial 1999 EU ban on the use of antibiotic growth promoters, but before the introduction of the 2006 legislation. Further research will investigate different interventions using prebiotics/probiotics to reduce the carriage of antimicrobial resistance in food producing animals (**Defra VM02203**).

4.5.3 Research to determine those aspects of the food production process (from farm to consumer) that represent a risk for the development of antibiotic resistant bacteria include studies on the repeated use of antibiotics in farm animals (**Defra OD2015**) and on the relationship between such resistance and a variety of medication and farming practices (**Defra OD2006**). In the latter project, antibiotic-resistant bacteria were found in all 25 farms investigated, including those where antibiotics had not been used for many years, if at all (and including 13 organic farms). Occurrence of ciprofloxacin-resistant *E. coli* was positively associated with use of fluoroquinolones in pig farms. It was also found that those farms with the highest overall use of antibiotics, including growth promoters, were positively associated with the highest levels of detection of resistant bacteria. Modelling of the occurrence and spread of antibiotic resistance is being used to identify management and husbandry techniques which are associated with high levels of antibiotic resistance in order to develop improved practices (**SEERAD SAC/254/00**). Wider risk assessment studies include consideration of the issues of antibiotic resistance, for example research addressing a risk based approach to the control of *Salmonella* in pig farms (**Defra OZ0323**).

4.5.4 In a study to determine the prevalence of *Salmonella* and *Campylobacter* in chickens for retail sale, it was found that over half the

Salmonella isolates tested were resistant to at least one antibiotic, with 23% of isolates showing resistance to four or more unrelated compounds. Over 40% of *Campylobacter* spp were resistant to at least one antibiotic, but multiple drug resistance was seen in fewer than 2% of all isolates (**FSA B18002**).

4.5.5 A further source of antibiotic resistant food-borne bacteria which may also present a risk to humans is the consumption of contaminated food (often leading to food poisoning) during foreign travel. Two studies have been supported which include examination of the risks foreign travel may offer to the acquisition of antibiotic resistant bacteria (**FSA B10004, B14001**).

4.6 Specific antimicrobial agents

Fluoroquinolones

4.6.1 A significant number of research projects have been supported which address the specific issue of the use of fluoroquinolones in farm animals, the development of associated antibiotic resistant bacteria (in particular *Campylobacter)* and the risks to human health through food-borne transmission of these antibiotic resistant bacteria. Fluoroquinolones are routinely used in the management of animal health as well as being important antibiotics in human health.

4.6.2 All the research projects funded by MSFFG member organisations and focussed on fluoroquinolones have examined some aspect of the development of antibiotic resistance to these or other agents in response to the use of quinolones. Many are discussed in earlier sections in this document: here it is appropriate to consider the overall findings of the research.

4.6.3 Several of the projects examined the impact that use of fluoroquinolones has on the incidence of antibiotic resistance (**Defra OD2004, OZ0501, VM02100, VM02101**). The general observation was that the use of fluoroquinolones did lead to the often rapid development of resistance in bacterial populations, including multiple antibiotic resistance. It has also been found that fluoroquinolone resistance can occur in response to the use of other antimicrobial agents and this issue is being further investigated (**Defra OD2004, OD2010, VM02200**). It was also observed that resistant strains of *Campylobacter* persisted for up to four weeks after the removal of fluoroquinolones (**Defra OZ0501**). Research is being undertaken to determine whether different dosing regimes of fluoroquinolones for poultry can be used to reduce the incidence of antibiotic resistance (**Defra VM02201**). Further investigations will determine if pharmacokinetic-pharmacodynamic modelling can be used to design rational dosage schedules that minimise the emergence of antimicrobial resistance (**Defra VM02204**).

4.6.4 Unexpectedly, in pigs at slaughter no strains of *C. jejuni* resistant to nalidixic acid or the fluoroquinolone ciprofloxacin were isolated (**Defra**

VM02110).

4.6.5 Evidence was gathered that at least some of the bacterial strains resistant to fluoroquinolones were found in both humans and animals (**FSA B10001**) and further work is being undertaken in this area (**FSA B10004**). It was also found that as far back as 1995, 13% of *Campylobacter* spp isolated from humans were resistant to fluoroquinolones (**DH 226**).

Other non-antibiotic agents

4.6.6 There are several groups of chemicals which are antimicrobial in effect some of which are used in cleaning and disinfection regimes. These include disinfectants, dyes and some organic solvents. Questions have been raised as to the potential effect of using these compounds on the resistance of bacteria to more conventional antibiotics, including fluoroquinolones and the development of multiple drug resistance.

4.6.7 It was found that in *Salmonella* there is a clear link between resistance to organic solvents and low level resistance to a number of antibiotics, whereas no such links were found in *Campylobacter* (**Defra OD2004, VM02100, VM02101**). It was also found that *Salmonella* were statistically more likely to develop multiple antibiotic resistance if the bacteria had been grown in the presence of disinfectants prior to growth in the presence of MAR-inducing antibiotics. In this case a similar result was observed with *Campylobacter*. Recent research explores the on-farm relevance and impact of these observations, with a view to understanding the (human) clinical significance of the mutants arising from this selective pressure (**Defra OD2010**). Other research focuses the possibility that the use of antibiotics which select for *Campylobacter* with the MAR phenotype may also lead to the same bacteria showing increased resistance to disinfectants (**Defra VM02200**).

5. Gaps in currently funded research

5.1 This is a major and complex area of research. The overall problem, that of the development of antibiotic resistance in bacteria that colonise and infect farm animals and humans, is of significant importance to human and animal health. Within this very large area of concern and research, the issue of the possible transfer of antibiotic resistance is a minor component and the body of international research addressing it is relatively small. However, the UK input in this area is one of the most significant internationally and considerable research effort has been funded by Defra and other MSFFG member organisations to determine how important the link is. The outcome has been that while there is evidence of transfer of zoonotic antibiotic resistant pathogens such as *Salmonella* from farm animals to humans through the food-supply chain (ACMSF (1999)) there is no evidence to suggest that the food chain represents a major risk for human infection with other

clinically relevant antibiotic resistant bacteria. The nature and extent of the research funded so far suggests that a part of the problem is actually in determining how to investigate the link, so as to avoid confounding factors and to give clear, quantified results. Studies to investigate the epidemiology of antibiotic resistance could address some of the problems, as could research to obtain a greater understanding of the association between veterinary antibiotic use and the occurrence of antibiotic resistant bacteria on farms and in farm animals.

5.2 Several research projects have been funded to investigate the effect of withdrawal of antibiotic growth promoters on the development of antibiotic resistant bacteria of significance both to farm animals and humans. There are also similar projects examining the impact of other changes in farming practice arising from the reduction in use of antibiotics. This is in general a relatively recent area of research, and there is likely to be a need for more extensive studies, in particular determining the impact of disinfectants and similar materials on the development of multiple antibiotic resistance in common food-poisoning bacteria. There may also be a need for a greater understanding of the relationship between resistance to specific antibiotics and other, unrelated, ones.

5.3 An emerging issue is the occurrence and spread of extended spectrum β lactamases (ESBLs) in a number of different bacterial species, including food-borne pathogens. There is a lack of detailed knowledge of the epidemiology of these bacterial species, including their occurrence in farm animals and the food supply chain.

5.4 Although not a food-borne pathogen as such, there is considerable concern about the possible acquisition by *S. aureus* of multiple drug resistance by any route. There are a variety of unknown issues in this area, such as the frequency of occurrence of MRSA in farm animals, whether there can be transfer of these bacteria to humans and whether there is transfer of resistance genes from animal *S. aureus* to human strains. Some research is supported in this general area, but there may be a need for further studies to address these questions.

5.5 The occurrence of vancomycin resistant enterococci, which have been identified as an emerging pathogen, and are of concern also for the risk of transfer of vancomycin resistance to *S. aureus*, has been identified as requiring further study in order to determine their presence and numbers in food (**FSA B08008**).

5.6 The occurrence of antibiotic resistant bacteria in imported food is an issue that has not been addressed. Research on this subject would be valuable.

5.7 The development of alternative antibiotic agents, such as lantibiotics (**BBSRC 0042222**) is an important area of research, not only in relation

to possible food safety issues. More research in this area could provide very wide benefits in human and animal health.

6. CONCLUSIONS

6.1 There is substantial evidence that the use of antibiotics in both humans and animals leads to an increase in antibiotic resistant bacteria, and that the frequency of occurrence of antibiotic bacteria in animals and humans has increased significantly in the last thirty years. This is accompanied by an increase in the spectrum of antibiotic resistance in bacteria (House of Lords Select Committee on Science and Technology (1998)). In this context, there is a concern that the food-supply chain may provide an opportunity for antibiotic resistance to be transferred from farm animals to humans, to the detriment of the latter.

6.2 The research described in this report emphasises in a number of ways the difficulty and complexity of the subject. There are few results that clearly point to a link between the occurrence of antibiotic resistant bacteria in animals and their transfer to humans. Some research does suggest this link, but it is generally difficult to obtain quantitative measures of the importance of this route of transmission to human health.

6.3 Whilst there is no overwhelming evidence that the food supply chain contributes significantly to the occurrence of the majority of clinically relevant antibiotic resistant bacteria in humans, there are a number of issues that will merit further research, in order to control and minimise the risk .

REFERENCES

Aarestrup FM, Syfarth AM, Emborg H-D, Pedersen K, Hendriksen RS and Bager F. (2001) Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrobial Agents and Chemotherapy **45** 2054-2059

ACMSF (1999) Report on microbial antibiotic resistance in relation to food safety. The Stationery Office, London ISBN 0 11 322283 1

Everett MJ and Piddock LJV (1998) Mechanisms of resistance to fluoroquinolones. In: Handbook of Experimental Pharmacology: Quinolone antibacterials. Eds Kuhlmann J, Dalhoff A and Zeiler H-J. Heidelberg, Springer-Verlag **127** 264

House of Lords Select Committee on Science and Technology (1998) Resistance to antibiotics and other antimicrobial agents. The Stationery Office, London.

Khachatourians GC (1998) Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Canadian Medical Association Journal **159** 1129 - 1136

Levy SB (1992) Active efflux mechanisms for antimicrobial resistance. Antimicrob Agents Chemother B36B 695 - 703

GLOSSARY

Antibiotic
A substance, produced by or derived from a microorganism, which selectively destroys or inhibits the growth of other microorganisms. Because compounds such as sulphonamides and quinolones are synthesised chemically, they are not strictly speaking antibiotics. However, in practice the term "antibiotic" often encompasses such agents and this broader view has been adopted in this report (ACMSF (1999))

Antibiotic resistance
The ability of a micro-organism to withstand an antibiotic.

AFLP
Amplified Fragment Length Polymorphism. Molecular markers typical of a strain of an organism obtained by combining RFLP and PCR techniques and applying these to restriction fragments obtained from a digest of an organism's total genomic DNA

Avoparcin
A glycopeptide antibiotic related to vancomycin, the antibiotic of last resort when treating methicillin-resistant *Staphylococcus aureus.* Avoparcin was used in animal husbandry until its ban by the EU in 1999 because of the risk of development of vancomycin resistance in *S. aureus.*

Biovar
A variant prokaryotic strain that differs physiologically and/or biochemically from other strains in a particular species.

Brucella abortus
The bacterium associated with brucellosis in cattle.

Campylobacter coli* and *Campylobacter jejuni
The two species of bacterial pathogen responsible for causing the majority of *Campylobacter* infections in England and Wales. *Campylobacter* is the most commonly reported bacterial cause of infectious intestinal disease in England and Wales in humans.

Ciprofloxacin
A fluoroquinolone antibiotic

DNA gyrase
A protein which relaxes DNA supercoils during DNA replication in bacteria.

DNA topoisomerase
An enzyme that alters the superhelix density in supercoiled DNA. This is necessary for DNA replication, when topoisomerases relax negatively supercoiled DNA, converting it to a less supercoiled form.

Efflux
Removal of antibiotics from within a bacterium by passage across the cell membranes.

Enterococcus
Bacteria normally found in the bowel of healthy people. They can cause illness such as urinary tract infections and bacteraemia. Strains which are resistant to glycopeptide antibiotics such as vancomycin have emerged.

Escherichia coli
A Gram-negative non spore-forming bacillus commonly found in the intestinal tracts of humans and other warm-blooded animals. *E. coli* is widely used in biology, both as a simple model of cell biochemical function and as a host for molecular cloning experiments. In environmental studies, it is a key indicator of water pollution due to human sewage effluent.

***E. coli* O157**
A serotype of *E. coli* associated with serious gastrointestinal disease in humans.

Erythromycin
A macrolide antibiotic. Other examples include tylosin and spyramycin.

Extended spectrum beta lactamases (ESBLs)
ESBLs are enzymes that confer upon their host organism the ability to resist a wide range of therapeutic beta-lactam antimicrobials (i.e. penicillins and cephalosporins).

Fluoroquinolones
A subset of the quinolone family of antibiotics, the parent molecule of which is nalidixic acid. They act by inhibiting DNA gyrase and therefore DNA replication and transcription. As quinolones are synthesised chemically, they are not strictly antibiotics but antimicrobials. However, common usage generally includes quinolones as antibiotics.

Growth promoters
Substances, including antibiotics, used to stimulate an animal's growth, mainly by improving daily liveweight gain and feed conversion efficiency.

Lightcycler™
An advanced PCR platform that allows quantitative detection of PCR products.

Listeria monocytogenes
Listeria spp. are Gram-positive non-sporing rods which have been known to cause human disease for many years. They are ubiquitous in the environment and found world-wide**.** *Listeria monocytogenes* is the species of *Listeria* most commonly found associated with human disease.

Macrolide antibiotics
A group of antibiotics, such as erythromycin and tylosin, that are similar in their structure, action and antimicrobial spectrum. They are characterized by having a large lactone ring that contains anywhere from 14-20 C atoms. They produced by various strains of *Streptomyces* . Their mode of action is to inhibit protein synthesis in the target bacterium.

Multilocus sequence typing (MLST)
An unambiguous procedure for characterising isolates of bacterial species using the sequences of internal fragments of seven house-keeping genes.

MSRA
Methicillin resistant *Staphylococcus aureus*

PCR
The Polymerase Chain Reaction. A technique used extensively to generate multiple copies of a target DNA sequence by amplification.

Pulsed Field Gel Electrophoresis
This technique separates DNA molecules by subjecting them to alternately pulsed, perpendicularly placed electrical fields and is used as a sub-typing tool.

Quinolones
Broad spectrum antibacterials which are chemically synthesised. The mode of action is to inhibit bacterial DNA gyrase (topoisomerase II) and topoisomerase IV. They are used in the treatment of acute diarrhoeal disease, including *Salmonella* and *Campylobacter* infections.

RFLP
Restriction Fragment Length Polymorphisms. Variation in DNA sequence that is easily recognized because it occurs at a site where a restriction enzyme cuts a specific sequence, producing DNA fragments of varying lengths. RFLPs often serve as genetic markers and are used to distinguish between different subtypes of bacteria.

***Salmonella enterica* serovar Typhimurium (*S.* Typhimurium)**
A ubiquitous Gram negative bacterium which causes food poisoning in humans.

***S.* Typhimurium DT104**
In the late 1990s, the serotype of *S.* Typhimurium most commonly associated with gastrointestinal illness in humans.

Serotype
Subdivisions of a (bacterial) species identified by their antigenic characteristics

Staphylococcus aureus
A Gram-positive bacterium that is a common coloniser of human skin and mucosa. It can cause disease, particularly if there is an opportunity for the bacteria to enter the body. Illnesses such as skin and wound infections, urinary tract infections, pneumonia and bacteraemia (blood stream infection) may then

develop. It can also cause food poisoning. Most strains of this bacterium are sensitive to many antibiotics, and infections can be effectively treated. Some *S. aureus* bacteria are resistant to the antibiotic methicillin, termed methicillin-resistant *Staphylococcus aureus* (MRSA).

Sulphonamides
A class of antibiotics that contain sulphur.

Vancomycin
A glycopeptide antibiotic used as a last resort in treating MSRA.

APPENDIX 1: PROJECTS FROM THE MSFFG DATABASE USED IN THIS REPORT

Project Code	Title	Funder	Contractors	Start Date	End Date
D15925	Transfer of newly identified antibiotic resistance genes between bacteria in different gut and non gut environments	BBSRC	University College London	Feb-2002	Jun-2005
0042222	Development and exploitation of novel antimicrobial genes	BBSRC	Institute of Food Research	Apr-2005	Apr-2007
772	Evolutionary consequences of the control of *Salmonella* in chickens	BBSRC	Institute for Animal Health	Oct-1998	Sep-2001
4311218	Bacterial Evolution, Antibiotic resistance and *in vivo* gene transfer	BBSRC	Institute of Food Research (IFR)	Apr-2000	Mar-2002
OD2001	An in vivo poultry model to study the effect of growth promoters in resistance to human antibiotics in mixed bacterial populations	Defra	University of Bristol	Oct-2000	Sep-2003
OD2002	Field investigation of acquisition and persistence of multiple resistant *E. coli* in cattle and sheep exposed to farm waste	Defra	Veterinary Laboratories Agency	Apr-2000	Mar-2004
OD2003	The effect of antimicrobial treatment and withdrawal on the population dynamics of enteric bacteria expressing resistance in the pig	Defra	University of Bristol	Jan-2001	Dec-2004
OD2004	Loss of antibiotic resistance: analysis of phenotype and related gene expression	Defra	Veterinary Laboratories Agency	Apr-2000	Mar-2003
OD2005	A laboratory and field study to assess the potential for transfer of antibiotic resistance between bacterial strains in stored and spread organic wastes	Defra	Central Science Laboratory	Apr-2000	Mar-2003
OD2006	Investigation of persistence of antimicrobial resistant organisms in livestock production	Defra	Veterinary Laboratories Agency	-Oct-2000	Sep-2005
OD2007	Genetic characterisation of resistance markers in sentinel *Escherichia coli* and *Enterococcus* in farm animals	Defra	University of Bristol	Apr-2001	Sep-2004

Project Code	Title	Funder	Contractors	Start Date	End Date
OD2008	Transfer of antimicrobial resistance genes between bacteria in stored and spread farm wastes	Defra	Veterinary Laboratories Agency	Sep-2000	Aug-2003
OD2009	Wild rodents as reservoirs of antibiotic resistance for farm animals and man-current status, origins and mechanisms of persistence	Defra	University of Liverpool	Oct-2000	Sep-2003
OD2010	Use and abuse of non-antibiotic antimicrobials as major contributors toward the development of antimicrobial resistance	Defra	Veterinary Laboratories Agency	Oct-2003	Sep-2006
OD2011	Development of proteomic targeted tests for microbial multiple antibiotic resistant zoonotic food borne pathogens	Defra	Veterinary Laboratories Agency	Jan-2003	Mar-2007
OD2012	Antibiotic resistance in animal bacteria and its transmission to clinical bacteria	Defra	University of Edinburgh	May-2004	Apr-07
OD2013	The potential for veterinary antibiotic use to cause glycopeptide resistance in human *Staphylococcus aureus*	Defra	St George's Hospital Medical School	Oct-2003	Mar-2008
OD2014	Effect of tetracycline use in animals on appearance and dissemination of resistance to third generation tetracyclines	Defra	Rowett Research Institute	Oct-2003	Mar-2008
OD2015	Antimicrobial resistance: an evaluation of risk factors to identify control strategies	Defra	University of Bristol	Jan-2004	Dec-2006
OD2019	Methicillin-resistant *Staphylococcus aureus* in companion animals and associated with the human healthcare sector	Defra	Royal Veterinary College	Sep-2005	Aug-2007
OD2020	MRSA in cattle - an investigation into selected properties of isolates recovered from clinical veterinary diagnostic samples	Defra	Veterinary Laboratories Agency	Jan-2006	Jan-2008
OD2022	Characterisation of antibiotic resistance gene silencing in *Escherichia coli*	Defra	University of Bristol	Jan-2006	Dec-2008
OZ0132	Antibiotic Resistance mechanisms in *Salmonella* and *Campylobacter*	Defra	Veterinary Laboratories Agency	Apr-1997	Mar-2000
OZ0323	An integrated risk based approach to the control of *Salmonella* in UK pig farms	Defra	Veterinary Laboratories	Apr-2005	Oct-2008

Project Code	Title	Funder	Contractors	Start Date	End Date
OZ0501	Antibiotic treatment of commercial broiler flocks: incidence and mechanisms of fluoroquinolone resistance in *Campylobacter*.	Defra	Agency University of Birmingham	Jan-2000	Mar-2003
OZ0502	In vivo models to investigate the development of antibiotic resistance	Defra	Veterinary Laboratories Agency	Apr-2000	Mar-2002
VM02100	Factors influencing the development of resistance to fluoroquinolone antibiotics by food borne bacteria	Defra	Veterinary Laboratories Agency	Apr-2000	Sep-2003
VM02101	Assessment of factors influencing the development of resistant to fluoroquinolone antibiotics used in pigs and poultry.	Defra	University of Reading	Jul-2000	Sep-2003
VM02103	Proactive health management - the influence of antibiotic usage in pigs	Defra	Royal Veterinary College	Aug-2000	Mar-2003
VM02105	Identification and use of genomic markers of antibiotic resistance in campylobacters, salmonellae and enterococci.	Defra	Veterinary Laboratories Agency	Aug-2000	Jul-2003
VM02110	An epidemiological study of the prevalence of bacteria resistant to antimicrobials in pigs at slaughter	Defra	Veterinary Laboratories Agency	Jan-2000	Jun-2001
VM02136	Development of rapid response gene profiling for identification of antimicrobial resistance genes in enterobacteria from food animals and humans	Defra	Health Protection Agency	Mar-2003	May-2006
VM02200	Antibiotic treatment of commercial broiler flocks: effect upon *Campylobacter* prevalence and resistance	Defra	University of Birmingham	Oct-2003	Sept-2006
VM02201	Modulation of dosing regimes to prevent development of fluorquinolone resistance in bacteria	Defra	Veterinary Laboratories Agency	Apr-2004	Mar-2006
VM02205	Genoprofiling of multiresistant *Salmonella enterica*	Defra	Health Protection Agency, Veterinary Laboratories Agency	Jun-2006	May-2007

Project Code	Title	Funder	Contractors	Start Date	End Date
VM0292	Protocol for field studies to monitor the persistence of resistance for up to 12 months after the withdrawal of antimicrobial feed activities	Defra	University of Glasgow	Oct-1999	Sep-2002
VM0294	Use of antibiotic compounds in food producing animals for therapeutic, prophylactic and/or growth promotion purposes	Defra	EntecUk Ltd	Sep-1999	Dec-2000
VM02203	Interventions to reduce the carriage of antimicrobial resistance in food producing animals	Defra	Veterinary Laboratories Agency	Apr-2006	Nov-2007
VM02204	Minimising the emergence of resistance to antimicrobial drugs through rational dosage schedule design based on pharmacokinetic-pharmacodynamic (PK-PD) modelling and population PK-PD modelling	Defra	Royal Veterinary College	Jul-2006	Jun-2010
226	Estimation of the prevalence of quinolone resistance in gastrointestinal pathogens	Department of Health	Health Protection Agency (HPA)	Mar-1995	Feb-1996
240	Molecular epidemiology of multiresistant *Salmonella typhimurium* DT104 in England and Wales	Department of Health	Health Protection Agency (HPA)	Apr-1996	Apr-1998
00-RESR086	A comparative study of thermophilic *Campylobacter* isolates of clinical, food and pet origin	Food Safety Promotion Board	University College Dublin and others	Mar-2001	Sep-2004
B08003	A study of the prevalence of verocytotoxin producing *E. coli* O157 in the Sheffield area	FSA	Health Protection Agency (HPA)	Apr-1997	Apr-1999
B08008	Methods used for the assessment of the number and prevalence of *Salmonella* and *Campylobacter* spp. in chicken on retail sale	FSA	Health Protection Agency (HPA)	Nov-1999	Jun-2000
B10001	Molecular epidemiology of multiple drug resistance and resistance to fluoroquinolone antibiotics in *S*. typhimurium DT104 and related phage types from humans and food animals	FSA	Health Protection Agency (HPA)	Jan-1999	Sep-2001

Project Code	Title	Funder	Contractors	Start Date	End Date
B10002	Drug resistant screening of *Campylobacter* isolates from IID study	FSA	Health Protection Agency (HPA)	Jan-2000	Mar-2000
B10003	Establishment of a national reference facility for antibiotic resistant bacteria from foods; development of a national archiving facility	FSA	Health Protection Agency (HPA)	Apr-2001	Mar-2003
B10004	Assessment of, relative to other pathways, the contribution made by the food chain to the problem of quinolone resistance in microorganisms causing human infections	FSA	Veterinary Laboratories Agency	Apr-2002	Mar-2005
B14001	Food poisoning and foreign travel - a study of population health burden and risk factors for antibiotic resistance	FSA	University of Wales – College of Medicine	Apr-2002	Mar-2005
B18002	UK-wide survey of *Salmonella* and *Campylobacter* in fresh and frozen chicken on retail sale	FSA	ADAS	Mar-2001	Jun-2001
G0300020	Development of a multipurpose DNA microarray for the identification and active surveillance of bacterial pathogens	MRC	London School of Hygiene and Tropical Medicine	Apr-2004	Apr-2007
G0501415	Unravelling multiple antibiotic resistance in *Salmonella enterica*	MRC	University of Birmingham	Jul-2006	Jul-2009
SAC/137/97	Effect of diet and husbandry practices on the selection of antimicrobial resistance in the normal enteric microflora of healthy pigs and food products derived from pork.	SEERAD	Scottish Agricultural College	Apr-1997	Mar-2000
SAC/147/97	Antimicrobial resistance of *Enterobacteriaceae* from farmed cattle and sheep.	SEERAD	Scottish Agricultural College	Apr-1997	Mar-2000
SAC/254/00	Antimicrobial resistance modelling	SEERAD	Scottish Agricultural College	Apr-2000	Mar-2004

Appendix 2: Report on the 8th Congress of Chemotherapy and Infection (ECC8) & 4th European Conference on Viral Disease (ConVir4), 26-27 October 2006

Summary

1. This primary theme of the conference was antimicrobial resistance: the mechanisms underlying this phenomenon and spread, the current levels of antimicrobial resistance in various bacterial species, the change in this across Europe and the clinical consequences of antimicrobial resistance. Parallel sessions on viral diseases were also held, including one on gastrointestinal viruses.

2. The material presented was tailored for an audience of medical professionals and aimed to present the latest advances in these fields coupled with the practical aspects of integrating this information into clinical practice.

Detail

3. This conference was organised by the Federation of European Societies for Chemotherapy and Infection and was attended by several hundred delegates, the majority of which were from across Europe but there were delegates from Australia, USA, Asia and the Middle East.

Points of Note

4. There were a number of expert lectures on the fundamental mechanisms of antibiotic resistance. A State of the Art lecture highlighted the importance of modulation of gene expression in antibiotic resistance. By only expressing resistance in the presence of antimicrobial agents organisms decrease the fitness cost associated with carriage and expression of these mechanisms leading to increased persistence of resistant organisms. Bacteria have a variety of highly sophisticated mechanisms for controlling expression of antimicrobial resistance determinants, the majority of which are only just being elucidated and understanding these may eventually lead to new strategies for controlling these organisms.

5. A symposia on multi-drug resistant bacteria covered how these emerge, how resistance mechanisms are accumulated and the clinical consequences of this. The importance of horizontal transfer of resistance as opposed to mutation and the effect this has on using predictive modelling to estimate spread of resistance was highlighted as was the loss of diversity in multi-drug resistant bacteria. The loss of diversity suggests that antimicrobial usage is selecting out lineages in a wide range of microorganisms that are particularly well adapted to uptake and maintenance of antimicrobial resistance determinants.

6. There were a number of symposia that covered issues relating to MRSA. The emergence and significance of glycopeptide resistance, measures for control and the consequences of inappropriate therapy for the patient were the main themes. The increase in MRSA displaying partial or full glycopeptide resistance and the difficulties in identifying these organisms were discussed in a number of presentations. It appears that these may be more prevalent than current reporting suggests because the presence of these organisms (particularly those displaying decreased susceptibility) may be masked due to difficulties with current laboratory methods. The ineffectiveness of vancomycin as the primary treatment for MRSA infections was highlighted. This is primarily related to the bacteriostatic nature of the drug and data has shown outcome is often poor even if the organism is sensitive to vancomycin.

7. There were a variety of oral papers devoted to fluoroquinolone resistance. The emphasis was on Gram negative organisms and resistance in *Campylobacter*, *Salmonella* and *E. coli* as well as the relationship with extended spectrum beta-lactamase (ESBL) production in Enterobacteriaceae was covered. Newer plasmid mediated mechanisms of fluoroquinolone resistance were discussed in detail – particularly the molecular basis of resistance and the level of resistance conferred.

8. The problem of movement of antimicrobial resistance was covered in a symposium devoted to trans-national antimicrobial resistance issues. There were presentations covering the role of the European Centre for Disease Prevention and Control and the antimicrobial resistance programme it runs, the problems with transfer or movement of patients from clinical settings across national borders and a presentation on imported food and international travel as risk factors for antimicrobial resistance in the EU. The latter presentation emphasised the difference in resistance rates between countries without and within the EU and highlighted the difficulties this would bring with regard to movement of patients and foodstuffs from countries with high levels of antimicrobial resistance to those with low levels.

9. ESBLs were covered in a number of symposia, oral papers and posters highlighting the emergence and significance of this mechanism of resistance. An expert lecture covered the clinical problems caused by organisms that produce ESBLs and concentrated on the clinical implications of infection with these organisms, improved methods for detecting them and ways of controlling them. A strong theme that came out of the conference was the increasing use of the carbapenems to treat these infections and there were a number of presentations detailing increasing levels of carbapenem resistance. Other presentations looked at multi-drug resistance in ESBL producers as well as treatment failure and there were numerous posters recording the rise of ESBL producers in healthcare settings across the EU. The emergence and spread of the CTX-M genotype of ESBL producers across Europe was particularly striking.

10. Efflux pumps, their importance in contributing to a multi-drug resistance phenotype and their induction or inhibition by non-antibiotics was covered in two sessions. Targeting these mechanisms as a novel route for controlling

zoonotic pathogens was explored in a presentation by researchers from the Republic of Ireland.

11. A session on gastrointestinal viruses covered the European surveillance of noroviruses and hepatitis E virus in Hungary. A presentation by Dr. Koopmans (Netherlands) detailed the background to the European Foodborne Viruses Network (http://www.eufoodborneviruses.co.uk/) and covered the work undertaken by the network in tracking and identifying new norovirus variants. The Hungarian study on hepatitis E was particularly interesting as the virus has been detected in a variety of wild and domestic animals in Hungary and human infection has been documented with a strain very similar to that found in pigs. A case report was discussed in which a human infection had been traced to a home-made cured pork sausage.

Conclusions

12. This conference provided a useful perspective of the problems associated with antimicrobial resistance, primarily in the healthcare setting. Persistence of problem organisms such as MRSA and relatively newly emergent organisms such as ESBL producing Enterobacteriaceae and the clinical challenges these present were covered in considerable detail.