safefood

The Problem of Antimicrobial Resistance in the Food Chain

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Abbreviations

AMR	Antimicrobial Resistance
AMRAP	Antimicrobial Resistance Action Plan
aw	Water Activity
CDAD	*Clostridium difficile* Associated Disease
CDC	Centres for Disease Control and Prevention
CDSCNI	Communicable Disease Surveillance Centre Northern Ireland
DAEC	Diffuse adhering
DEFRA	Department of Environment, Food and Rural Affairs
DNA	Deoxyribonucleic acid
EaggEC	Enteroaggregative
EARSS	European Antimicrobial Resistance Surveillance System
ECDC	European Centre for Disease Control
EFSA	European Food Safety Authority
EIEC	Enteroinvasive
ELISA	Enzyme-linked immunosorbent assay
EPEC	Enteropathogenic
ESAC	EU Surveillance of Antibiotic Consumption
ESBL	Extended Spectrum ß-Lactamases
ETEC	Enterotoxigenic
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organisation
FSAI	Food Safety Authority of Ireland
GMOs	Genetically Modified Organisms

GRAS	Generally Regarded as Safe
H_2S	Hydrogen Sulphide
HPSC	Health Protection Surveillance Centre
HUS	Haemolytic ureamic syndrome
IFT	Institute of Food Technologists
LAB	Lactic Acid Bacteria
MAR	Multiple Antibiotic Resistance
MATE	Multi-drug and Toxic Compound Extrusions
MDR	Mutli-Drug Resistant
MF	Major Facilitator
MIC	Minimum Inhibitory Concentration
MIC	Minimum Inhibitory Concentration
MLSB	Macrolides, Lincosamides and Streptogramins
MRS	Methicillin-Resistant Strains
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NDSC	The National Disease Surveillance Centre
NI	Northern Ireland
NRL	National Reference Laboratory
OIE	World Organisation for Animal Health
QAC	Quaternary Ammonium Compound
QACs	Quaternary Ammonium Compounds
RNA	Ribonucleic acid
RND	Resistance-Nodulation-Cell Division
ROI	Republic of Ireland
SARI	Strategy for the Control of Antimicrobial Resistance in Ireland

SE	Staphylococcal enterotoxin
SMR	Small Multi-drug Resistance
UK	United Kingdom
VRE	Vancomycin Resistant Enterococci
VTEC	Verocytotoxigenic *Escherichia coli*
WHO	World Health Organisation

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...resistant bacteria can be transmitted from food-producing animals such as cattle, pigs and poultry, and the environment to humans, via the food chain.

Executive Summary

Background

Antimicrobial resistance (AMR) associated with the food chain is currently a subject of major interest to many food chain stakeholders. In response ***safe*food** commissioned this report to update our knowledge of this area and to raise awareness of the issue. Its primary focus is on the food chain where it impacts consumer health. This review will inform and underpin any future action to be taken by ***safe*food** with regard to AMR.

What is antimicrobial resistance?

Antibiotics are important drugs used in the treatment of bacterial infections in both humans and animals. Emerging antibiotic resistance among certain bacteria is now frequently observed, thereby posing a serious threat to public health. Once these micro-organisms become resistant to one or more antibiotics, they do not respond to therapy.

Some bacteria are naturally resistant, whilst others become resistant following selection after prolonged antibiotic use. Reservoirs of resistant bacteria may develop in the gastrointestinal (GI) tracts of food-producing animals following mis-use of these valuable therapeutic drugs. These resistant bacteria can be transmitted from food-producing animals such as cattle, pigs and poultry, and the environment to humans, via the food chain.

In this report, emphasis has been placed primarily on those bacteria of animal origin that infect humans via the food chain. Specifically this report reviews the scientific data available in relation to *Salmonella* spp., verocytotoxigenic *Escherichia coli* (VTEC), *Campylobacter* spp., all of which are important zoonotic bacteria. The report summarises information on other AMR bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* that may have a food-borne role that has yet to be defined. Importantly, the document comments on commensal bacteria, which inhabit the GI tracts of animals and humans, these have hitherto been an unrecognised reservoir of resistance. Consideration is also given to the role of AMR in probiotic bacteria and genetically modified organisms (GMO).

What causes bacteria to develop AMR and why is this important for food safety?

It is likely that the emergence of AMR in bacteria involves a complex series of events in humans, animals and the environment, occurring over an extended period. In the past AMR has been recognised in clinical and veterinary settings alone, and would never have been linked with the food chain. We derive much of our food from farmed animals, a feature that links human and animals alike. The food chain and the broader environment provide a convenient route, by which humans can become infected with AMR bacteria.

Emerging AMR: the potential exposure routes

(1) Food-producing animals & the environment

AMR arises following the mis-use of antibiotics. Humans are exposed to or acquire AMR bacteria through selection pressure associated with prolonged use of these important drugs. Furthermore, humans may also be exposed through direct contact with animals and humans that may be colonised/infected with AMR bacteria. A lesser recognised route however, is the food chain which may contain AMR bacteria derived from food-producing animals or from cross-contamination during food processing.

In the environment, AMR bacteria can enter the food chain through the contamination of ground and surface water, or from the spraying of food crops with contaminated water containing AMR bacteria derived from human and animal waste. Where these crops are not processed further after harvest, this food source may pose an increased risk to public health.

(2) Food processing technologies

Food processing technologies are designed to reduce the risk of transmission of hazards including bacteria through the food chain. As bacteria become resistant, following the stress imposed through antibiotic selection, they may evolve and undergo genetic changes which make these organisms more difficult to eliminate. This may in turn increase the likelyhood of transmission through the food chain.

(3) Use of sanitizing agents & biocides in food production

Sanitizers are used in the food industry to eliminate contaminating bacteria that may occur on food preparation surfaces or equipment in direct contact with food. Although sanitizers, biocides and antibiotics kill bacteria, there is increasing concern that resistance to these cleaning agents may directly or indirectly be linked to AMR. Concerns have been expressed in regard to the application of sanitizers and biocides in food processing and in domestic food preparation environments where they may promote the development and dissemination of AMR via the human food chain. Currently there is a need to develop a better understanding of this relationship (if any).

Human Health Impact

There are a range of human health consequences from AMR including protracted illness and even death. Vulnerable patients with impaired immunity are at greatest risk.

...the primary focus of this report is on food and food processing technology with particular impact on the consumer-health aspects.

Strategies to reduce the emergence of AMR bacteria

On the island of Ireland there are two AMR strategies currently active, the Strategy for the control of Antimicrobial Resistance in the Republic of Ireland (SARI) and the Anti Microbial Resistance Action Plan (AMRAP) in Northern Ireland. Both SARI and AMRAP recommended the development of guidance protocols in relation to the appropriate use of antimicrobials (in humans), as well as strategies to monitor the supply and use of antimicrobials in our hospitals and the community. Education awareness programmes have been developed which are focussed on medical professionals, veterinary practitioners and the general public. These aim to create a better understanding of AMR and how it links to our broader society. Managing and controlling any future emergence of AMR micro-organisms will require a multi-disciplinary approach with imputs from all health care professionals and stakeholders in the food industry.

Recommendations

Based on the current ***safe*food** report the following recommendations were offered in support of the surveillance infrastructure and research requirements:

Surveillance:

- Intensify AMR surveillance in the animal population, across the food chain, and in the human population
- Integrate the all-island AMR Monitoring Programme (including prescribing, dispensing and consumption patterns in human and animal populations)
- Promote the prudent use of antimicrobial agents in animal and human medicine
- Establish a forum on AMR and food safety, including all stakeholders to insure delivery of the surveillance strategy.

Research:

- Improve our understanding of the impacts of AMR on current and alternative food processing technologies
- Food attribution studies should be undertaken to determine the fraction of food animal-associated AMR infections on the island of Ireland and to assist with the identification and prioritisation of hazards posed.

Background and terms of reference of the report

Background:

There is already considerable activity and information on antimicrobial resistance (AMR) at the beginning and end of the human food chain i.e. veterinary and clinical. However, the primary focus of this report is on food and food processing technology with particular impact on the consumer-health aspects. This theme is appropriate to the remit of ***safe*food**. This area is under explored, although there is some current scientific literature on the relationship between AMR, modern food production and consumer/food interaction. This review will inform and underpin any action to be untaken by ***safe*food** on AMR and food safety.

Terms of reference of the report:

- The prevalence of AMR bacteria (pathogens and commensals) in the food chain
- The impact of food processing stresses on the survival and evolution of AMR bacteria within food; and the transfer of AMR among bacteria within the food matrix
- The impact of currently used antimicrobials including disinfectants, sanitizers, surfactants and related decontamination treatments
- The human health impact of antimicrobial use in food animals

...widespread use of antibiotics led to the emergence of antibiotic resistance in many important pathogens...

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Introduction

What are antibiotics?

Antibiotics are low molecular weight microbial metabolites that can kill or inhibit the growth of sensitive bacteria. The term 'antibiotic' refers to drugs used to treat infectious diseases in animals, humans and plants and these substances may be naturally occurring, semi-synthetic or synthetic. Most of the drugs in use today were discovered between the years 1940 and 1970. Chemical modification of the structure of antibiotics leads to the development of new compounds with an altered spectrum of activity. The therapeutic benefit of antibiotics depends on their selective toxicity.

Widespread use of these drugs led to the emergence of antibiotic resistance in many important pathogens (Levy, 2002). This resistance contributes to higher rates of morbidity and, in the case of severe bacterial infections, therapeutic failure (Masterton *et al*., 2006).

What is antimicrobial resistance?

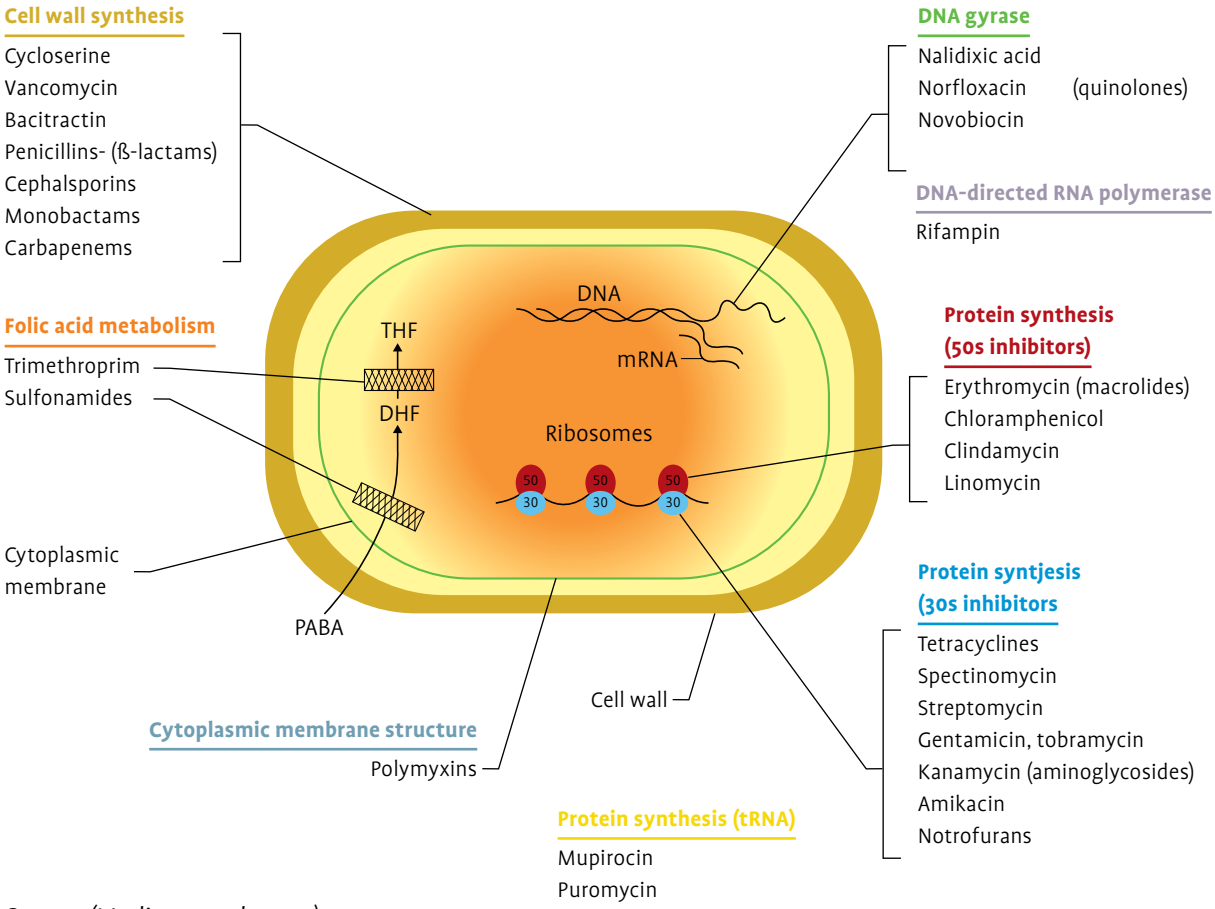
Definition of resistance

The development of antimicrobial resistance can be seen as a global problem in microbial ecology and is the best-known example of a rapid adaptation of bacteria to a new ecosystem (Carattoli, 2001). Micro-organisms are ubiquitous in nature and there is a continuous exchange of genetic information between bacteria inhabiting diverse ecological niches including, humans, animals and the broader environment. Genetic exchanges, combined with the selection of partially-resistant microorganisms through continued exposure to sub-lethal doses of antibiotics, contribute to the emergence of groups of intermediate resistant and/or (clinically) resistant bacteria. The position of clones on the susceptible/intermediate/resistant continuum is generally defined on the basis of in vitro parameters, related to their ability to survive exposure to a defined concentration of an antimicrobial compound. Bacterial infections can be described as being clinically resistant if they have a low probability of responding to

drug treatment, even if the maximum dose of the antimicrobial compound in question is given (EUCAST, 2000; Acar and Rostel, 2001). Degrees of antimicrobial susceptibility/resistance in clinical isolates are often defined in terms of the Minimum Inhibitory Concentration (MIC) of an antimicrobial compound required to prevent bacterial growth.

Bacteria can be defined as being resistant to an antimicrobial compound, when its MIC is higher than its wild-type counterpart (EUCAST in 2000; Acar and Rostel 2001). For this reason, the 'epidemiological cut-off values' will vary among different species and geographical regions. Antimicrobial drug resistance can be defined under a number of headings as follows. A summary of the mode of action of some antibiotics is illustrated in Figure 1.

Figure 1 Mode of Antibiotic Action



Source: (Madigan et al. 2000)

Intrinsic resistance

Intrinsic resistance relates to the unique physiological properties of a micro-organism, in which their metabolic activity is substantially unaffected by the presence of an antimicrobial compound. For example, a bacterium may not possess a particular metabolic pathway, or internal structure, normally targeted by the antimicrobial compound.

Such resistances are generally chromosomally encoded, and are typically responsible for observed differences in resistance observed between genera, species and strains of bacteria. Intrinsic resistance can be associated with differences in cell wall structures, the ability to pump antimicrobial compounds out of the bacterial cell using efflux pumps, or the production of enzymes capable of inactivating antimicrobial compounds within the bacterial cell (Russell, 2001; Gilbert and McBain, 2003). Gram-negative bacteria in general have a higher resistance to antimicrobials than Gram-positive bacteria (Russell and Chopra, 1996). More specifically, Gram-negative bacteria are intrinsically resistant to penicillin G, a ß-lactam antibiotic, by virtue of their different structure, which prevents the antibiotic from accessing the cell wall. Intrinsic resistance is not considered a clinical problem because antibiotics were never intended for use against these resistant bacteria.

Acquired resistance

Acquired resistance, in which a previously sensitive bacterium becomes resistant, can arise as a result of (a) a mutation in the organisms' Deoxyribonucleic acid (DNA), or (b) through the acquisition of one or more antimicrobial resistance genes as a result of horizontal gene transfer within and between bacterial species.

(a) Mutation in the organisms DNA

De novo emergence i.e. a mutation in the chromosomal DNA of a house-keeping structural or regulatory gene (Courvalin and Trieu-Cuot, 2001) occurs approximately once in every one billion cell divisions (Pallasch, 2003). These mutations are associated with uncorrected errors arising during DNA replication. Although rare, such mutation events occur all too frequently against the background of the millions of on-going bacterial replication processes in our environment. The vast majority of these errors are disadvantageous to the host bacterium, and do not persist at cellular or population levels. However, the smaller number of such genetic mistakes which are advantageous to the host bacterium can persist and, through a process of natural selection, emerge to confer resistance to many bacteria, presenting significant problems in clinical medicine.

Emergence of antimicrobial resistance can be accelerated by several means. Thus, different members of a bacterial species will mutate at different rates (Martinez and Baquero, 2002), e.g. approximately one per cent of *E. coli* exhibit a hypermutation phenotype (LeClerc *et al.*, 1996; Matic *et al.*, 1997). The proportion of hypermutable strains is much higher in chronic infections, including cystic fibrosis, chronic stomach infections (Bjorkman *et al.*, 2001) and chronic obstructive pulmonary disease (Macia *et al.*, 2005).

(b) Horizontal gene transfer

Once antimicrobial resistance-conferring genes have appeared by mutation, they can be mobilised between strains and species by a processes of horizontal gene transfer. These processes, which can occur in the environment (in vitro) or during infection (in vivo), include conjugation, transduction and transformation, and can involve one or more defined genetic elements including: bacteriophages, plasmids, conjugative transposons and integrons (Carattoli, 2001). The detailed mechanisms of these processes are outlined later in this document and this could be appropriately regarded as a dynamic process wherein a pool of resistance genes is being actively shared across the prokaryotic kingdom.

In line with this view, the term 'resistome' has been coined recently to include all antimicrobial resistance-encoding genes present in the environment that are potentially transferable to pathogenic bacteria (D'Costa *et al.*, 2006). Therefore, it is important to recognise that many environments contain a wide range of non-pathogenic micro-organisms carrying antimicrobial resistant determinants, can potentially be transformed to sensitive pathogenic bacteria under appropriate conditions. This means that efforts to limit the acquisition of antimicrobial resistance by human pathogens must recognise the much wider resistance repertoire that may be encountered in sewage facilities, animal and human microbiota (Martinez *et al.*, 2007), foods and the food processing environments (Mc Mahon *et al.*, 2007; Walsh *et al.*, 2008).

Multi-drug resistance

Antimicrobial compounds are divided into different chemical classes (Figure 1), with each class containing a group of closely related compounds or their chemical derivatives, which all act in a similar manner in susceptible bacteria. Multi-drug resistance is defined as resistance to three or more individual classes of antimicrobial compound.

Cross-resistance and cross-selection

Cross-resistance occurs when a single biochemical mechanism confers resistance to more than one member of a group of related antibiotics (i.e. a class). Conversely, antibiotics belonging to different classes are structurally dissimilar, having different cellular targets and are, therefore, usually not subject to cross-resistance. As a consequence, resistance is a class-based phenomenon since it affects the impact of antibiotics of a particular group, but not antibiotics belonging to other classes. However, cross-resistance between various antibiotic classes can also occur by two mechanisms, i.e., overlapping targets and increased drug efflux action (Courvalin and Trieu-Cuot, 2001).

Once antimicrobial resistance-conferring genes have appeared by mutation, they can be mobilised between strains and species...

Overlapping targets

Macrolides, lincosamides and streptogramins (MLS_B, including streptogramin B) are chemically, if quite distantly related (Depardieu and Courvalin, 2001). However, constitutive methylation of a single adenine base in ribosomal RNA confers a high-level of resistance to all three classes of antibiotics (Fluit *et al.*, 2001). Clinically, the MLS antibiotics are used primarily for the treatment of a variety of Gram-positive infections, especially methicillin-resistant staphylococci (Zhanel *et al.*, 2001). The increasing appearance of MLS-resistant strains has compromised their treatment options in recent years and as a result many synthetic derivatives of the macrolides have had to be produced.

Antibiotic efflux

A common mechanism in bacteria is an active export by membrane-bound efflux transporters (Poole, 2005; Piddock, 2006; Poole, 2007), by which bacteria eliminate antibiotics and other structurally unrelated chemicals, including dyes, bile salts, anticancer and antifungal drugs (Neyfakh, 2002). Antimicrobial resistance in over-expressing efflux mutants can be attributed to two mechanisms, (a) an increase in the expression of efflux pump activity, or (b) through amino acid substitution(s), making the pump protein more efficient at exporting xenobiotic compounds (Piddock, 2006). Efflux also delays the death of the bacterium by lowering the intracellular antibiotic concentration (Yu *et al.*, 2003), thus providing the bacterium with additional time for mutation to occur (Courvalin and Trieu-Cuot, 2001). Drug-specific efflux pumps have also been identified and these are usually associated with mobile genetic elements, which can be transferred between bacteria. In contrast Multi-Drug Resistant (MDR) efflux pumps are generally chromosomally encoded (Poole, 2007). The broad substrate specificities of these efflux pumps accounts for the decreased susceptibility in some bacteria, to ß-lactams, aminoglycosides, chloramphenicol, fluoroquinolones, MLS_B drugs, tetracycline, trimethoprim, sulfonamides and other chemical compounds. It is well-established that MDR efflux pumps confer clinically relevant resistance to antimicrobial compounds in bacteria (Poole, 2005).

Hazard identification

Antimicrobial resistance can be considered a direct hazard when the aetiological agent involved in infection is an antibiotic resistant food-borne pathogen. An indirect hazard arises though the transfer of resistance genes to another bacterium such as a commensal or pathogenic organism.

Relevance to food producing animals and human health

Until recently antimicrobials have been added to feed and water to promote growth and limit infection in food-producing animals. Although, such supplementation poses minimal risk in terms of toxicity to the animal, it disturbs the normal animal flora. The risks associated with the development of antimicrobial resistance are increasingly recognised as significant (WHO, 2008). A recent meeting between the Food and Agriculture Organisation (FAO), the World Organisation for Animal Health (OIE) and the World Health Organisation (WHO) (Geneva in 2003) concluded that the emergence of antimicrobial resistant organisms associated with non-human usage of antimicrobials posed adverse consequences to human health. More recently, new European Union (EU) regulations banning the use of antibiotics as growth promoters in animals have been introduced in an attempt to reduce the emergence of antibiotic resistance in the food-chain.

...the emergence of antimicrobial resistant organisms associated with non-human usage of antimicrobials poses adverse consequences to human health...

Such policies are in accordance with the WHO recommendations and Codex Alimentarius Commission guidelines. Many classes of antimicrobial compound(s) are currently used in both human and animal therapy, raising concerns about the possibilities of the development of resistance in animals, and the direct (contact) or indirect (food) transfer of such resistance to humans. Such concerns, coupled with the lack of newly developed antimicrobial drugs, have led to the necessity to establish management systems, to contain and if possible, prevent antimicrobial resistance. Regulatory measures such as restriction or banning the use of antimicrobial compounds for specific purposes, or in specific animal species, have also been established.

Two expert meetings convened by the WHO (2005, 2007) resulted in a classification system for antimicrobial drugs. Antimicrobial drugs were determined as 'critically important', 'highly important' or 'important' on the basis of the extent to which they were sole therapies:

(i) one of a limited number to treat serious human disease and
(ii) used to treat diseases caused by micro-organisms that may be transmitted via non-human sources or diseases caused by microorganisms that may acquire resistance genes from non-human sources (see appendix for Table 1.)

Resistant organisms, pathogenic to humans, have been recovered from food animals or their derived products...

2

Antimicrobial agent use in food animals as a cause of human illness

It has been postulated for some time that the use of antimicrobial compounds in food animals may contribute to the emergence of antimicrobial resistant-related health problems in humans (Bates, 1997). This hypothesis involves a number of causation steps a) conditions that facilitate antimicrobial resistance b) emergence of antimicrobial resistance in an organism pathogenic to humans c) human exposure to the resistant organism d) host susceptibility to the organism and e) virulence changes associated with antimicrobial resistance.

There is on-going evidence supporting the hypothesis through each of the stages individually or across multiple stages. These are as follows:

Causative steps in antimicrobial resistance

Conditions that facilitate antimicrobial resistance

Antimicrobial agents are widely used in food animal production for therapeutic and prophylactic use, as well as for growth promotion (Shea *et al.*, 2004). The use of sub-therapeutic doses in healthy animals for prophylaxis and growth promotion, coupled with imprecise dosages given to ill and healthy animals (by delivery of antimicrobials through feed or water), facilitates antimicrobial resistance though selection (Hamer, 2002; Wegener, 2003; Shea *et al.*, 2004; Rosengren *et al.*, 2008).

Emergence of antimicrobial resistance in an organism pathogenic to humans

Resistant organisms, pathogenic to humans, have been recovered from food animals or their derived products, including:

- Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from cows milk (Leonard and Markey, 2008)
- MRSA in pigs (Armand-Lefevre et al., 2005, Huijsdens et al., 2006)
- resistant *Salmonella* spp. on egg-shells (Little et al., 2006)
- resistant *Salmonella* spp. in cow's milk (Murphy et al., 2008) and in retail meats (including chicken, pork, turkey and beef) (White et al., 2001
- Chen et al., 2004) resistant *E. faecium* in chickens and pork (Sorenson et al., 2001); multi-drig resistant *Campylobacter* spp. in feedlot cattle (Minihan et al., 2006) and
- ß-lactam-resistant *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* in food animals (Li *et al.*, 2007)

In the Netherlands the incidence of fluoroquinolone resistant *Campylobacter* spp. increased from 0 to 14 per cent in broiler chickens, and from 0 to 11 per cent in farm workers, following the introduction of enrofloxacin and saxifloxacin on poultry farms (Hamer, 2002).

The emergence of Vancomycin Resistant enterococci (VRE) (Kayser, 2003) was attributed to the use of avoparcin, a growth promoter chemically related to the glycopeptide antibiotic vancomycin. In some instances the use of one antimicrobial compound may give rise to the development of a MDR phenotype, as several resistance genes may be linked and transferred together on a mobile genetic element, (Shea *et al.*, 2004; Li *et al.*, 2007).

Human exposure to the organism

Humans can be exposed to antimicrobial-resistant bacteria through food derived from colonised animals, through increasing antimicrobial resistance in the environment and through food contamination during processing. Environmental contamination with resistant organisms, of surface waters for example, has also been reported, giving rise to concerns about direct exposure (Shea *et al.*, 2004). Numerous outbreaks of resistant infections associated with food animal sources have been reported, with some examples provided in Table 2:

Table 2 Human exposure to resistant bacteria

Country	Reference	Organism	Numbers affected	Study type	Main observations	Comments/ Limitations
The Netherlands / International	(Wulf et al., 2008)	MRSA	N/A	Prevalence study of MRSA carriage in veterinarians	272 participants at an animal health conference were screened and the rate of carriage of MRSA was 12.5%, but the prevalence from some countries was higher: Italy (61%); Germany (33%); Netherlands (23%); Spain (18%); Belgium and France (16%)	Zoonotic source of MRSA may contribute to increasing Community Acquired MRSA (CA-MRSA) incidence
The Netherlands	(van Loo et al., 2007)	MRSA	35 with NT-MRSA (not previously seen in Netherlands) 49% hospitalised	Case control study	Geographical location of cases linked to density of pig farms. Epidemiological association with contact with pigs (link to contact with cattle too)	Association with tetracycline use in pig and cattle farming reported

Humans can be exposed to AMR bacteria through food from colonised animals, antimicrobial resistance in the environment and food contamination during processing.

The Netherlands	(Huijsdens et al., 2006)	MRSA	2 ill	MRSA investigation following child hospitalised	6 humans associated with pig-farm colonised. 8 of 10 pigs MRSA positive Identical strains	Netherlands MRSA control programme uses “search and destroy” and these findings raise concerns about new source of MRSA
The Netherlands	(Voss et al., 2005)	MRSA	N/A	Prevalence study of MRSA in pig farmers compared with general Dutch population	6/26 (23%) of pig farmers screened were colonised with MRSA showing increased prevalence of MRSA as compared with the general Dutch population	More evidence of pig farming as a new source of MRSA, which might threaten the effectiveness of MRSA control programmes in the health sector

Europe – Iceland, England & Wales, The Netherlands, Scotland and Germany	(Crook et al., 2003)	*Salmonella* Typhimurium DT204b	International outbreak investigations – case control studies in two countries	International outbreak investigations – case control studies in two countries	In Iceland, link with imported lettuce. History of S. Typhimurium DT204b associated with bovine antibiotic use, and decreased ciprofloxacin sensitivity found associated with fluoroquinolone use in agriculture	No phage typing in Iceland. Late identification of outbreak as international. No analytical epidemiology in some of the countries and no overall outbreak investigation. Enternet surveillance networks assisted recognition of outbreak

Numerous outbreaks of resistant infections associated with food animal sources have been reported.

Europe – Iceland, England & Wales, The Netherlands, Scotland and Germany	(Crook et al., 2003)	*Salmonella* Typhimurium DT204b	392 patients – 16% hospitalised (at least 11% – hospitalisation status not ascertained for all)	International outbreak investigations – case control studies in two countries	In Iceland, link with imported lettuce History of S. Typhimurium DT204b associated with bovine antibiotic use, and decreased ciprofloxacin sensitivity found associated with fluoroquinolone use in agriculture.	No phage typing in Iceland Late identification of outbreak as international No analytical epidemiology in some of the countries and no overall outbreak investigation Enternet surveillance networks assisted recognition of outbreak
Denmark	(Helms et al., 2002)	*Salmonella* Typhimurium. (susceptible and resistant)	953 pan-susceptible 1094 cases resistant	Matched cohort study	Cases with pan-susceptible *Salmonella* Typhimurium 2.3 x more likely to die within 2 years compared with general population; with increased risk of death to 10.3x for quinolone resistant *Salmonella* Typhimurium	Adjusted for co-morbidities Used population registers

England/ Wales	(*Campylobacter* Sentinel Surveillance Scheme Collaborators, 2002)	Ciprofloxacin resistant *Campylobacter jejuni*	>650 notified cases	Surveillance	Domestic cases associated with pre-cooked cold meats; cases with recent foreign travel history associated with consuming chicken	Enhanced surveillance data collection limited, so not all exposure questions answered
US- Nebraska	(Fey et al., 2000)	*Salmonella* – ceftriaxone resistant	Hospitalised child	Lab testing of faecal samples	Child's isolate the same strain as cattle samples from family / neighbour herds	No herd antibiotic use information available
Ireland	(Grein et al., 1999)	*Salmonella* Typhimurium DT104	58 cases 7 hospitalised	Retrospective cohort analysis	Epidemiological link with turkey – traceback found *Salmonella* Typhimurium DT104 in young turkeys in the originating poultry farm	Additional information about the contribution of S. Typhimurium to burden of disease – 54% of all cases, and 80% of these multiresistant

Denmark	(Molbak et al., 1999)	*Salmonella* Typhimurium DT104. Quinolone resistant	27 cases, 11 hospitalised, 2 died	Surveillance – Outbreak investigation	Outbreak linked to pork from 1 slaughterhouse, and to 2 linked swine herds microbiologically – PFGE identical strains. Link supported by exposure history of majority of cases Small number of occupational or nosocomial exposures	Quinolone resistance hampered treatment with some treatment failures, including in 1 patient who died
US – California	(Cody et al., 1999)	*Salmonella* Typhimurium DT104	31 Outbreak 1 79 Outbreak 2 13% hospitalised	Matched case control studies	Epidemiological link to raw milk cheeses – Mexican-style. Link to one dairy – incomplete investigation as second implicated diary never visited. Outbreak 2 only came to light during case finding for Outbreak 1	Limited laboratory resources for typing. Late environmental investigation hampered findings

US	(Glynn et al., 1998)	*Salmonella* spp. / *Salmonella* Typhimurium DT104	39,032 *Salmonella* spp. 3903 salmonellae in study	Surveillance; also National *Salmonella* antimicrobial resistance study	Of 1326 sent for typing, 25% of which were *Salmonella* Typhimurium of which 34% were multi-drug resistant. In study, 25% of 3903 were S. Typhimurium, and 28% of these were five drug resistant	Trends showed increasing proportion of *Salmonella* were multi-drug resistant. Issues of standardisation of typing /sub-typing discussed

Host susceptibility and host vulnerability to the organism

The normal host immune response offers considerable protection against many infections, but patients with compromised immune systems are more vulnerable to these illnesses. Extremes of age are a recognised risk factor associated with increased vulnerability, as are underlying illnesses, including chronic disease, and prior antimicrobial treatment (Shea *et al*., 2004). Other host factors may include: a long hospital stay, intensive care unit stay, having a urinary or vascular catheter; and antibiotic therapy (Kayser, 2003). Use of proton pump inhibitors was also found to be a risk factor for S. Typhimurium DT104 (OR11.2, 95% CI 3.9-31.9) (Doorduyn *et al*., 2006). No vaccines are currently available to protect humans against antimicrobial-resistant organisms, including *Salmonella* spp., *Campylobacter* spp. or MRSA (Chin, 2000).

No vaccines are currently available to protect humans against antimicrobial-resistant organisms...

Virulence changes associated with antimicrobial resistance

The development of an antimicrobial resistance has been linked with increased virulence in some pathogenic organisms. This observation may be explained by the fact that the genes encoding resistance and virulence are located on plasmids, or other transmissible elements (Foley and Lynne, 2008). The use of antimicrobial compounds may co-select for virulence in these cases (Molbak, 2005).

Enterococci form part of the normal gastrointestinal flora of animals and humans and some of these organisms are used in the manufacture of food or as probiotics. More recently, however, enterococci have emerged as important nosocomial pathogens with some strains being resistant to several antimicrobials. Following the introduction of avoparcin, a glycopeptide growth promoter (chemically related to and conferring cross-resistance to vancomycin) in Europe in 1974, resistance to this agent began to emerge among isolates recovered from animals, the food supply and urban and rural sewage supplies (Bager *et al*., 1997). This development is now linked with VRE in animals. These organisms can be transmitted from the intestinal tracts of animals to humans. Using molecular sub-typing approaches, an association has been demonstrated between the use of the growth promoter avoparcin in animals and VRE cases in humans.

Many antimicrobial resistant bacterial infections involve longer hospitalisations and increased mortality (Angulo *et al*., 2004). The Centers for Disease Control and Prevention (CDC) review of 2004 provided evidence of the association between the use of antimicrobial agents in food animals and human illness (Angulo *et al*., 2004).

Relationship between antimicrobial use in animals and human illness

The majority of the evidence acquired through outbreak and epidemiological investigations of sporadic infections, field studies, case reports, ecological and temporal associations and molecular sub-typing studies (Table 2) support the causal link between the use of antimicrobial agents in food animals and human illness. A few papers have questioned this but these have not survived detailed scrutiny.


Name
Hospital No.
Room No.

Micro-organisms have developed seven major mechanisms to evade the bactericidal or bacteriostatic actions of antibiotics.

3

Antimicrobial resistance

Mechanisms of antimicrobial resistance

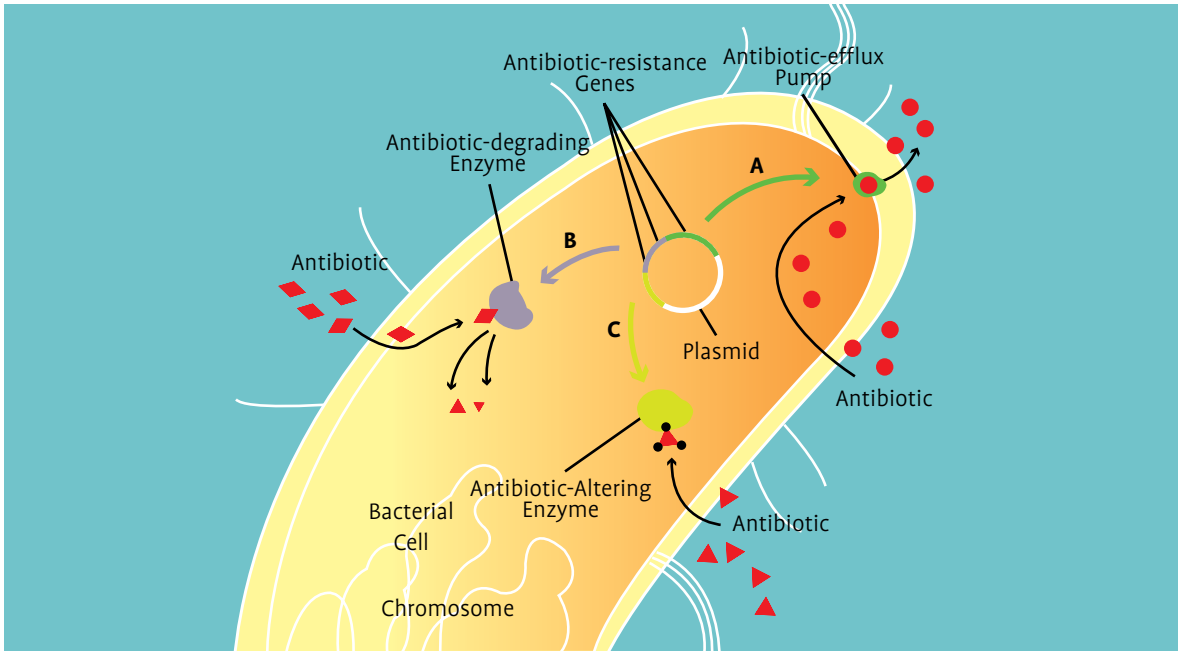
Micro-organisms have developed seven major mechanisms to evade the bactericidal or bacteriostatic actions of antibiotics. These mechanisms are listed in Table 3 and the main mechanisms are illustrated in Figure 2.

Table 3 Mechanisms of antibiotic resistance.
(Adapted from Pallasch, 2003)

Enzymatic antibiotic inactivation
ß-lactamases: ß-lactams (penicillins, cephalosporins) Acetyltransferases: aminoglycosides, chloramphenicol and streptogramins
Modification/ protection of target sites
Modified penicillin binding proteins: ß-lactams
Altered DNA gyrase and topoisomerase IV: fluoroquinolones
Altered RNA polymerase: Rifampin
Methylation of an adenine of 23S rRNA: erythromycin, clindamycin, streptogramins
Alteration of 16S rRNA: tetracyclines
Altered tetrahydrofolate and dihydrofolate reductase: sulfonamides and trimethoprim
Substitution of terminal peptidoglycan alanine with lactate: vancomycin and teicoplanin
Limiting antibiotic access to microbial cell
Altered outer membrane porins/reduced membrane transport: most antibiotics
Active efflux
Antibiotic efflux proteins: tetracyclines, fluoroquinolones
Failure to activate antibiotic
Decreased flavodoxin production: metronidazole
Development of alternate growth requirements
Production of auxotrophs: enterococci
Overproduction of target sites
Hyper ß-lactamase production: enteric bacilli

Horizontal gene transfer may occur by various mechanisms including conjugation, transduction and transformation.

Figure 2 Mechanisms of microbial resistance to antibiotics



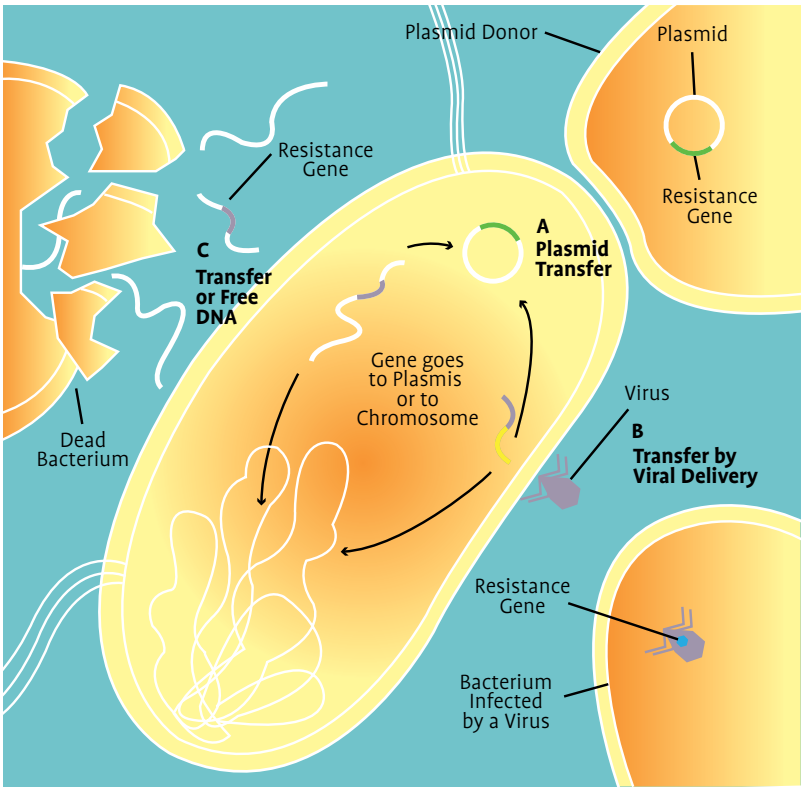
Adapted from: (Levy, 1998), a) efflux pumps, b) antibiotic degrading enzyme and c) antibiotic altering enzyme

Mechanisms of transmission-Horizontal gene transfer

Transfer may occur by various mechanisms including conjugation, transduction and transformation (see Figure 3). In addition, the contribution of transposable elements and integrons linked to plasmids has been reported (Carattoli, 2001). Transposons and integrons accelerate the development of bacterial resistance by assisting in the accumulation, expression and dissemination of resistant genes.

These features support the onward transmission of antibiotic resistance genes to daughter cells by replication or transfer within or among other bacterial species, typically by conjugation (Carattoli, 2003).

Figure 3 Transferable antibiotic resistance mechanisms in bacteria



Adapted from: (Levy, 1998), (a) conjugation, (b) transduction and (c) transformation

Plasmid

A plasmid is an extrachromosmal genetic element, which can maintain and express genes of interest, similar to the chromosome. However, the genes encoded on a plasmid are not usually present on the bacterial chromosome and typically confer a specialised function (i.e. an R-plasmid confers antimicrobial resistance), which can improve the cell's adaptability, survival and growth (Martinez and Baquero, 2002).

Plasmids also have the ability to multiply independently of the bacterial chromosome and, in many cases, contain the necessary information for their independent transfer from cell to cell, between and among different species and genera of bacteria (Wilson, 2000). Thus, the original host may retain some of the multiple plasmids, while one or more copies can be transferred to additional recipient host bacterial cells. Antibiotic resistance is carried on sections of plasmids called transposons, or jumping genes. Transposons are able to jump out of one plasmid

Antibiotic resistance is carried on sections of plasmids called transposons, or jumping genes.

and into another, carrying antibiotic resistance with them (Carattoli, 2003). They can also jump from a plasmid to the bacterial chromosome and back again. By this means, plasmids can accumulate resistance to an increasing number of antibiotics, which are then easily available to be transposed within and between bacterial species.

Conjugation

Conjugation requires the donor to have a particular type of plasmid or a transposon to mobilise the donor DNA, recognise/bind a suitable recipient and transfer the gene(s) of interest (Day, 1998). There are four different classes of plasmid-borne integrons that carry antibiotic resistance genes. Each integron carries a distinct integrase gene, of which class 1 integrons are the most common. The 3'-ends of class 1 integrons usually carry *sul1*, a gene coding for sulfonamide resistance. This class of integron can carry a varying number of up to nine gene cassettes. Class 2 integrons are different and usually carry three gene cassettes (*dfr1a, sat1, aadA1*), which include a *dfr* gene encoding trimethoprim resistance.

Co-trimoxazole resistance is reported to signal the presence of integrons (Leverstein-van Hall *et al.*, 2003). Strains with integrons have a higher chance of being resistant to aminoglycosides, quinolones and ß-lactams. Class 3 and 4 integrons have only been observed in a few cases in Japan and India, respectively. Integrons have the ability to capture genes, notably those encoding antibiotic resistance by site-specific recombination (Carattoli, 2003). Integrons have an integrase gene (*int*), a distal recombination site (*attI*) and a promoter (*Pant*), which flanks a central region wherein gene cassettes are recombined (Hier *et al.*, 2004). More than 60 different gene cassettes have been identified, with some integrons possessing multiple cassettes arranged in tandem and recombined in a classical *head-to-tail* orientation (Mazel *et al.*, 2000). As these resistance determinants are under the control of a single promoter all recombined gene cassettes are subsequently co-expressed. Thus, selective pressure for one determinant by the use of a particular antibiotic, will co-select for resistance determinants encoded by downstream cassettes. This is also important in light of the fact that integrons (and also transposons) have been shown to possess a number of genetic determinants encoding resistance to Quaternary Ammonium Compounds (QACs) and heavy metals (Olsen, 1999). This suggests the possibility that, exposing integron-carrying bacteria to sub-inhibitory levels of QACs, may co-select for antibiotic resistant bacteria in the absence of antibiotics (White *et al.*, 2003).

Transduction

Transduction is the movement of DNA from one bacterium to another via bacteriophages (bacterial viruses). This usually occurs when a virus picks up resistant DNA from one bacterial cell and injects it into another (Smyth *et al.*, 2003). Phage particles can protect DNA during transfer, as they generally show higher levels of resistance to adverse environmental conditions than free DNA (Day, 1998).

Transformation

Transformation is an active and dedicated process of gene exchange, governed by chromosomal genes that allow the uptake of exogenous free DNA by a competent cell (Smith *et al.*, 2003). Competence is often a transient and tightly-regulated process, through which a proportion of a population passes due to physiological changes occurring within those populations (Day, 1998). It is a mechanism of widespread, but not universal, gene exchange among bacterial strains (Wolfgang *et al.*, 1999). Many transformable bacteria release DNA during growth. Thus, at least 50 different bacteria have been demonstrated as competent to acquire genes released into the environment from other microbes, plants, yeasts and animals (Havarstein, 1998). Genes gained following transduction and/or transformation must be integrated into a plasmid or chromosome to be functional (Levy, 1998).

Effect of food processing technologies

The frequent isolation of MDR isolates from food sources, suggests that antibiotic resistant genes are present in a wide variety of different bacterial species found in food. This raises the question as to whether or not antibiotic resistance and sensitive bacteria respond differently to food processing stresses like heat and acid (Humphrey, 2001). Does antibiotic resistance confer cross-resistance to other food-related stresses, or does it impose a fitness cost, making antibiotic resistant cells more susceptible to such stresses? Increased resistance to low pH environments would raise concern about the stability and safety of dairy and fruit juice products. Increased resistance to thermal stress would be of concern in relation to a wide range of mildly heat-treated foods, in particular meat products. Accurate information on potential changes in response to food processing parameters is especially important when considering low infectious dose pathogens, such as, *E. coli* O157, where the presence of even a small number of cells could be clinically significant. Very little data is available in this area and the small number of reports available present conflicting results. This is unfortunate, as accurate information is essential to allow food processors to effectively eliminate undesirable pathogens from food, with minimum damage to the nutritional, organoleptic and visual properties of the product. Precise processing parameters may be necessary to gain the best possible balance between food safety and food quality.

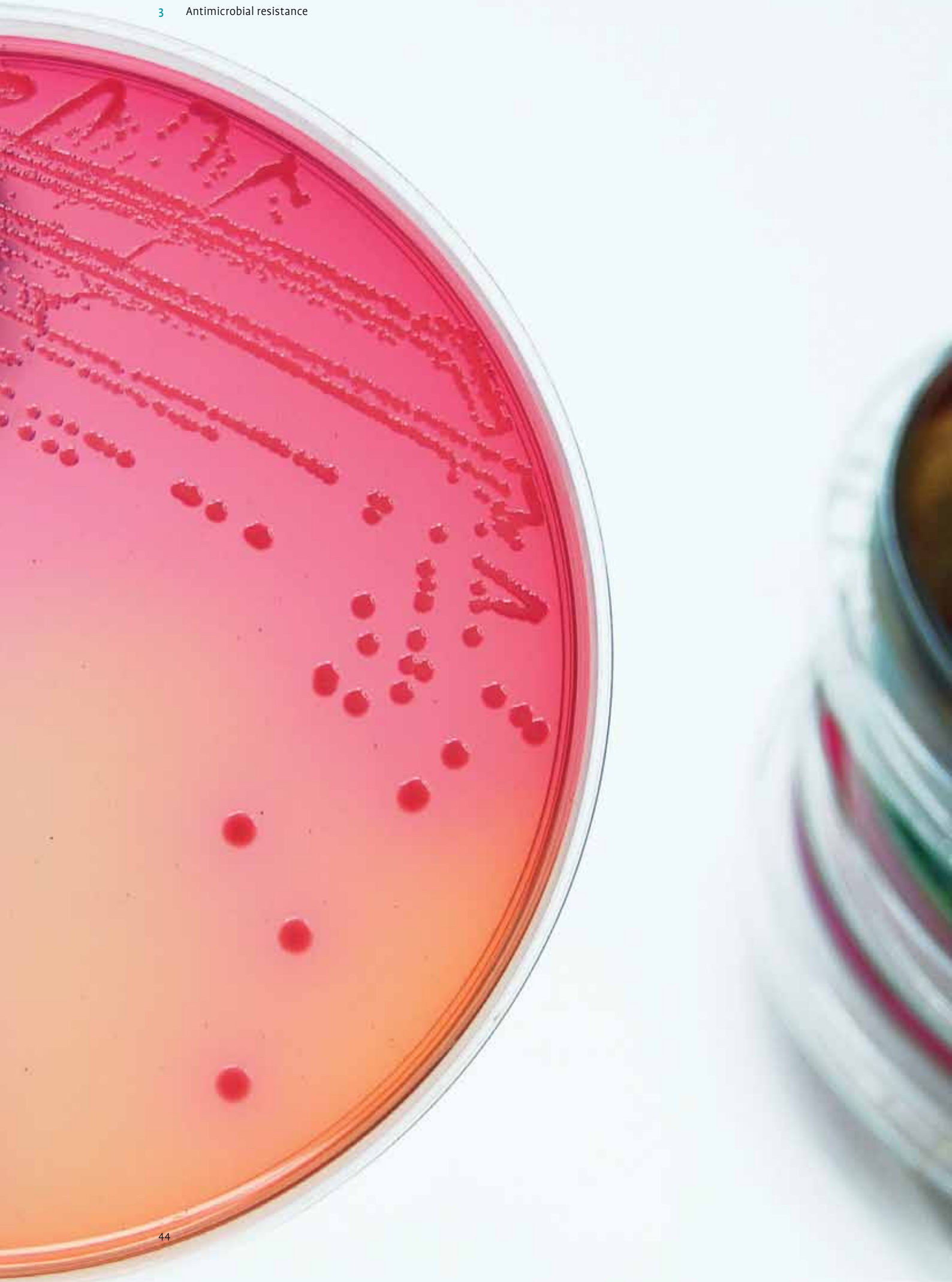
A further concern is that sublethal food processing stress could play a role in the development and persistence of antibiotic resistance/MDR, through the action of the Multiple Antibiotic Resistance operon or by increasing the rate of horizontal plasmid transfer (including MDR plasmids). For example, food processing-related stresses can cause increases in DNA damage, reductions in the efficiency of DNA repair and increases in membrane permeability. It is speculated that these stresses can result in the development of antibiotic resistance within the bacterial cell, including the release/transfer of antibiotic resistant determinants to other cells (Lado and Yousef, 2002). The current evidence for these concerns will be discussed in detail later in this report.

The frequent isolation of MDR isolates from food sources, suggests that antibiotic resistant genes are present in a wide variety of different bacterial species found in food.

Antimicrobial resistance and bacterial fitness

The development and/or acquisition of AMR enables host cells to survive in otherwise hostile (antimicrobial containing) environments. The more chromosomal and/or plasmid-borne genes the host carries, the more energy it has to expend in maintaining and replicating this *heavier* compliment of genes, making the host less efficient and generally less competitive compared to susceptible cells carrying fewer resistance genes. Such reductions in *bacterial fitness* can for example, reduce host growth rates, leading to overgrowth by *leaner*, antibiotic susceptible strains. However, slower growth is also related to increased resistance to other unrelated environmental stresses, including those used in food processing (McMahon *et al*., 2007). It is therefore possible that the increasing incidence of AMR may have significant implications for the food production and processing industry, by reducing the efficiency of food preservation techniques, and reducing current safety margins within the food industry. AMR-associated increases in resistance to common food processing interventions, such as the application of low/high temperature, water activity (a_w) reduction or pH modulation is of particular concern in the safe production and preservation of *minimally-processed* foods. Such foods, which form an expanding fraction of our total food supply, are welcomed by consumers as *fresh* and *natural*. However, these operate within fairly narrow margins of safety which could be breached by the slight increases in bacterial resistance to environmental stress which may be associated with the acquisition of a resistance marker. This means that accurate up-to-date information on antimicrobial resistance and their impacts on bacterial fitness are essential to assure safe food processing and preservation schemes.

A number of studies have reported that chromosomal AMR mutations, do not confer a fitness cost during growth (Gustafsson *et al*., 2003), heat treatment (Walsh *et al*., 2001; Duffy *et al*., 2006; Walsh *et al*., 2006), or at low pH (Tkalcic *et al*., 2000). However, other studies suggest that some chromosomal AMR strains do incur a small, but observable, fitness cost (Sander *et al*., 2002). Fitness costs have also been observed during growth (Blackburn and Davies, 1994; Gustafsson *et al*., 2003), heat treatment (Doherty *et al*., 1998) and survival at low pH (McGee, 2003) in a variety of different strains although such fitness costs may decline (partially or totally) in subsequent bacterial generations. For this reason fitness costs incurred during the initial development of resistance, may be rapidly reduced by compensatory mutation (Maisnier-Patin *et al*., 2002). Such compensation has been reported to occur in either *in vivo or in vitro* studies (Björkman *et al*., 2000; Reynolds, 2000). Other studies report compensation through a variety of secondary mutations, resulting in increased fitness (over-compensation), i.e. the emergence of organisms with enhanced survival characteristics (Reynolds, 2000) including superior growth rates (Ince and Hooper, 2003) and virulence (Bjorkman, 1998). The relationship between AMR and fitness will be discussed further for each foodborne pathogens in this report.



Salmonella enterica is a major cause of bacterial enteric illness in humans and animals...

4

Specific organisms

Salmonella – Major pathogenic bacterial hazards

Non-typhoidal *Salmonella*

Introduction

Salmonella enterica is a major cause of bacterial enteric illness in humans and animals and is notably the second leading cause of zoonotic infection in the European Union (Gupta *et al.*, 2004). Human infection can also be acquired through direct contact with carrier domestic or wild animals, or through consumption of contaminated foods.

Description of species

Salmonella spp. are Gram-negative, facultatively anaerobic rods, which are usually motile by peritrichous flagella. Their optimal growth temperature is 37˚C, during which D-glucose and other carbohydrates are metabolised with the production of acid and (usually) H2S gas. However, they do not utilise urea (Holt *et al.*, 1994). *Salmonella* spp. are oxidase negative, catalase positive, indole and Voges-Proskauer negative, methyl red and Simmons citrate positive (Madigan *et al.*, 2000). They are readily killed by heat and acid and are resistant to both freezing and drying, particularly in the presence of proteins and protectants. This genus prefers pH values between 4 and 8 and requires a water activity of 0.93 and above (Baird-Parker, 1990). They are found to occur in humans, warm and cold blooded animals, food and the environment. They are pathogenic to humans and many other animal species (Tietjen and Fung, 1995). The infectious dose can be as few as 15-20 cells, depending on age, health of the host and strain differences among the members of the genus. *Salmonella* spp. can cause typhoid fever, enteric fever, gastroenteritis and septicaemia (Holt *et al.*, 1994).

Salmonella in food on the island of Ireland

In 2006, 1.3 per cent of the raw poultry meat tested at the processing level was positive for *S. enterica,* followed by 5.3 per cent in 2007. This slight increase was the first observed since *Salmonella* spp. contamination rates were reported to be declining in 2001. This increase was not mirrored in poultry meat products, as

only a marginal difference (0.2%) was evident between 2006 (0.3%) and 2007 (0.5%). No contamination was reported at retail level in 2006 and very low levels of contamination (0.1%) were observed similarly in 2007 (Table 4). The most frequently isolated serotypes from poultry meat and associated products in 2006, were S. Agona (16%), S. Kentucky (15%) and S. Mbandaka (15%), followed by S. Kentucky (75%), S. Agona (10%) and S. Enteritidis (4%) in 2007.

S. enterica was not isolated from any of the 1,190 egg or egg products sampled in 2006. In 2007, one isolate (0.1%) of the 809 egg and egg products sampled, was found to be positive for *Salmonella* spp. (S. Agona) (Table 4).

S. enterica was identified in 1.7 per cent of raw pork and 0.5 per cent of pork products sampled at processing in 2006. Higher rates of recovery were observed at a processing level in 2007, as 2.9 per cent of raw pork and 0.7 per cent of pork products tested positive for *S. enterica* (Table 4). S. Typhimurium was the most frequently isolated serotype from pork and pork meat products in 2006 and 2007 (48% and 45% of all the serotypes identified, respectively) (FSAI Zoonosis Report 2009). A relatively low proportion (0.06 to 0.2%) of beef, veal and associated products were found to be contaminated with *S. enterica* at the processing level, compared to none at the retail level in 2006 and 2007 (Table 4). Of the *S. enterica* isolates recovered, S. Typhimurium (37% of serotypes in 2006 and 34% in 2007) and S. Dublin (37% of serotypes in 2006 and 20% of 2007) were the most predominant serotypes recovered in both years (FSAI Zoonosis Report 2009). In 2006 and 2007, a low incidence (0.01 to 0.8%) of *S. enterica* was recovered from other meat and meat products at the processing level, and none at retail. S. Typhimurium was found to make up 32 and 22 per cent of the *S. enterica* strains isolated in 2006 and 2007, respectively (Table 4).

Salmonella most commonly isolates from raw poultry meat compared to other meats.

Table 4 *Salmonella* spp. in food products in the ROI 2005

Food type	Sampling site	Tested 2006	Positive 2006	Tested 2007	Positive 2007
Poultry					
Raw poultry meat*	Processing level* Retail level	6,477 8	84 (1.3% 0)	5,976 4	319 (5.3%) 0
Poultry meat products	Processing level Retail level	6,698 1,378	22 (0.3%) 0	5,578 1,281	27 (0.5%) 1 (0.1%)
Eggs & Egg Products					
Table eggs		148	0	104	1 (0.1%)
Egg products		1042	0	1667	0
Pork & Pork Products					
Raw pork meat	Processing level	2,929	51 (1.7%)	2,015	58 (2.9%)
Pork meat products	Processing level Retail level	9,053 943	46 (0.5%) 1 (0.1%)	9,111 951	59 (0.7% 0 (0%))
Beef & Veal					
Raw beef /veal meat	Processing level Retail level	21,644 3	36 (0.2%) 0	23,003 38	27 (0.1%) 0
Beef/veal meat products	Processing level Retail level	13,783 491	25 (0.2%) 0	13,768 389	8 (0.06%) 0

Other meat & meat products					
Fresh Meat					
Sheep meat	Processing	1,506	2 (0.1%)	1,532	2 (0.1%)
Sheep meat	Retail	2	0	0	0
Unspecified meat	Processing	2,273	21(0.8%)	3498	13(0.4%)
Unspecified meat	Retail	2	0	0	0
Meat Products		781	0	782	0
Sheep meat	Processing	57	0	35	0
Sheep meat	Retail	5,540	1(0.01%)	5035	9(0.2%)
Unspecified	Processing	332	0	361	0
Unspecified	Retail				

(Source: FSAI, 2009)

Epidemiology of *Salmonella* spp. on the island of Ireland and in Europe

In 2007, there were 456 reported cases of salmonellosis (10.8 cases/100,000 population) in the Republic of Ireland (ROI), indicating a small increase in incidence from 2006 (10.0 cases/100,000 population) (HPSC, 2009). The highest age-specific incidence rate was in children 0-4 years of age (35.7 cases/100,000 population) with 24 per cent of all cases occurring in this age-group. As in previous years, cases peaked between July and October.

In Northern Ireland (NI), 159 cases of salmonellosis were confirmed in 2007 and 186 cases in 2008 (provisional data) (CDSCNI, 2009).

In Europe, 151,995 cases of salmonellosis were reported in 2007 (31.1 cases/100,000 population), showing a 7.3 per cent decrease from the previous year (EFSA, 2009). As seen for the ROI, the highest age-specific incidence rate was found in children aged 0-4 years of age (125.4 cases/100,000 population).

In Europe 151,995 cases of salmonellosis were reported in 2007, the highest age-specific incidence rate was found in children 0-4 years of age.

Serotypes

S. Enteritidis (n=179) and *S.* Typhimurium (n=114) remain the two most predominant serotypes (39% and 25% respectively) involved in human illness in the ROI in 2007. Other serotypes included; *S.* Newport (n=13), *S.* Kentucky (n=9), *S.* Typhi (n=8), *S.* Java (n=8) and *S.* Infantis (n=8). Eight isolates of *S.* Typhi and five of *S.* Paratyphi A isolates were detected in 2007, as opposed to seven *S.* Typhi isolates and one *S.* Paratyphi A isolate in 2006.

In NI (and the UK) the most common serotypes in human infection were *S.* Enteritidis and Typhimurium (EFSA 2006). In the EU, *S.* Enteritidis was reported at 65 per cent and *S.* Typhimurium at 17 per cent of cases (EFSA, 2009).

Food

The National Reference Laboratory (NRL) (Department of Agriculture Fisheries and Food, 2008) for *Salmonella* spp. typed 1,475 isolates of *Salmonella* spp. in 2007. These were recovered through official control programmes or submitted by private laboratories supporting food safety controls operated by food business operators. Of the isolates detected, *S.* Kentucky was the most prevalent (n=612), followed by *S.* Typhimurium (195), *S.* Derby (99) and *S.* Agona (93), with 26 strains of *S.* Enteritidis also being isolated.

However, the most recent UK wide outbreak of salmonellosis (148 cases to date, September 2008) was caused by *S.* Agona which was present in a range of contaminated sandwich fillers supplied by a single food company. This outbreak became Europe-wide, matching the distribution of contaminated material (www.hps.scot.nhs.uk).

The serotypes most commonly isolated from animals are species dependent (Table 5), with *S.* Dublin the most prevalent serotype in cattle, *S.* Diarizonae in sheep, *S.* Livingstone in chickens and *S.* Typhimurium in pigs. *S.* Rissen and *S.* Mbandaka are the serotypes most commonly isolated from animal feed-stuff, however *S.* Typhimurium, *S.* Enteritidis and *S.* Hafar have been isolated on a few occasions (DEFRA, 2006). Although the *Salmonella* spp. serotypes most commonly associated with human salmonellosis are not the most prevalent serotypes found in animals, the fact that pathogenic *Salmonella* spp. serotypes can be cultured from animals is a cause for concern and perhaps suggests that there may be a connection via the food chain or via animal contact.

In the EU, isolates from meat were commonly resistant to ampicillin, nalidixic acid, streptomycin and tetracycline with variations in the levels of resistance in food animals, being reported in different member states (EFSA, 2006).

Table 5 *S. enterica* serotypes present in UK animals and food. (Adapted from Ong et al., 2007)

Source	**Prevalent** *Salmonella* spp. **serotypes** (% isolates reported)	**% resistant to antibiotics**					
		Fully S[s]	**Te**	**Chl**	**3rd**	**Cip Ceph.**	**Amino glycocides**
Human infection	Enteritidis (68%) Typhimurium (20.8%)	72.3$	7-38%*	1-4%*		8-15%	0-98%*
Chicken UK	Livingstone (17.5%) Senftenburg (12.3%)	44%	22%	8%	0%	<1%	22%
Cattle UK	Dublin (60.5%) Typhimurium (21.5%)	73%	20%	18%	0%	0%	12%
Sheep UK	Diarizonae (71.4%) Typhimurium (8%)	92%	81%	47%	0%	0%	63%
Turkey UK	Typhimurium Derby	50%	39%	14%	0%	0%	31%
Pig UK	Typhimurium (66.2%) Derby (13.9%)	11%	81%	47%	0%	0%	63%
Retail eggs non-UK	Enteritidis (3.3%)						
Retail eggs UK	*Salmonella* spp (0.38%)						
Feedstuff	Rissen, Mbandaka						

Fully SS = susceptible to all antimicrobials examined

Data consolidated from DEFRA 2006, with additional information from

** AMR 2004*

The emergence of MDR in *Salmonella* spp. is considered an important evolutionary step in the epidemiology of salmonellosis.

Recent surveys for the presence of *Salmonella* spp. on raw meat products in NI indicated a high incidence of *Salmonella* spp. (40%) on post-eviceration pig carcasses (McDowell *et al*., 2007). A study of the prevalence of *Salmonella* spp. in the pork chain on the island of Ireland, reported that, *Salmonella* spp. was recovered from 24 of 720 (3.3%) pork cuts in boning halls of pork abattoirs in the ROI and from 44 of 525 (8.38%) of pork cuts in NI. The difference in prevalence in both jurisdictions was not found to be statistically significant. Furthermore, a study of the prevalence of *Salmonella* spp. on retail pork cuts found *Salmonella* spp. present in 13 of 500 (2.6%) pork samples purchased in butchers shops or supermarkets in ROI. In comparison, *Salmonella* spp. was recovered from 11 of 200 (5.5%) pork samples purchased in butchers shops or supermarkets in NI. S. Typhimurium (approximately 50%) and S. Derby (approximately 20%) were the most dominant serotypes.

A recent report noted that four per cent of raw chicken contained *Salmonella* spp. in retail raw chicken in NI, (Meldrum and Wilson, 2007). In contrast to an earlier study by Soultos *et al.* (2003), this report represents an increase of 1.5 per cent. The incidence in beef is unknown (no data available), although *Salmonella* spp. were recently isolated from three per cent of faeces from post-slaughter (healthy) cattle (Madden *et al*., 2007). No data is available on the incidence of *Salmonella* spp. in eggs in NI, although it is thought to be low with similar rates to those reported in the UK – a direct result of the widely-adopted flock vaccination regime. A recent survey of *Salmonella* spp. in eggs on the island of Ireland, reported only two isolates (S. Infantis, S. Montevideo on egg shells) recovered from 30,000 samples (Murchie *et al*., 2007), confirming that domestic (UK/Irish) produced eggs are currently unlikely to present a significant source of human salmonellosis.

Antimicrobial resistance in *Salmonella* spp.

Salmonella enterica continues to be an important cause of gastroenteritis with an increase reported in the ROI, NI, England and Wales in the year 2007. S. Typhimurium DT104 is perhaps the best-known and most widely studied serotype of *Salmonella* spp. because of its MDR phenotype. The emergence of MDR in *Salmonella* spp. is considered an important evolutionary step in the epidemiology of salmonellosis. Until recently S. Typhimurium DT104 could be distinguished from other *Salmonella* spp. isolates, by the presence of *Salmonella* genomic Island (SgI1). This genomic island, typically confers resistance to five antimicrobials (ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline; ACSSuT) and has the ability to capture and stably maintain additional genes, encoding other antibiotic resistance determinants. As the SgI1 is chromosomally located, it is stably maintained in the absence of selective pressure. This feature continues to drive the emergence of new variants of S. Typhimurium DT104 with reduced susceptibility to a larger repertoire of antibiotics. Moreover, the presence of the SgI1 is linked to higher rates of morbidity/mortality (Mulvey *et al*., 2006). Recent reports of the dissemination of SgI1 among other serotypes of *Salmonella* spp. and Proteus are alarming, particularly with regard to major pathogens of the developing world, such as, *S. paratyphi* (Velge *et al*., 2005). The presence of the SgI1 limits therapeutic options, particularly in the developing world where chloramphenicol

is the antibiotic of choice as it is cheap and broad spectrum.

Many S. Typhimurium isolates recovered in the ROI in 2007, were found to have a resistance profile typical of that conferred by the presence of the SGI-1 (63% resistant to ampicillin, 64% to sulphonamide, 71% to tetracycline, 61% to streptomycin and 42% to chloramphenicol). In comparison, for NI, there is no recent data available for antimicrobial resistant profiles.

Reports in the EU found similar resistance profiles (ACSSuT) in *Salmonella* spp., with additional resistance to the quinolones and nalidixic acid (EFSA, 2008).

MDR is prominent in the *Salmonella* spp. population in the ROI (see Table 6). Reports of antibiotic resistance in clinical samples in the ROI confirms that MDR resistance in S. Typhimurium is a growing problem, with resistance to trimethoprim and nalidixic acid also emerging. Similar resistance profiles are appearing in other serotypes (S. Stanley and S. Typhi), mostly likely as a result of transfer of the SgI1 (see Table 6).

Table 6 AMR of human S. *enterica* serotypes isolated in the ROI 2006. (Adapted from EPI-Insight, 2007a)

	% Of Isolates Resistant to Antibiotics						
Serotype (No. Isolates)	**AMP**	**CHL**	**STREP**	**SULPH**	**TET**	**TRIM**	**NAL**
Enteritidis (158)	8	0.6	2	2	0.6	0.6	0
Typhimurium (101)	69	58	64	68	66	6	6
Agona (5)	20	0	20	40	20	0	40
Virchow (10)	30	20	30	60	50	60	100
Hadar (11)	45	0	100	18	100	18	45
Stanley (6)	33	0	50	50	50	33	0
Typhi (7)	43	43	43	43	0	43	57
Kentucky (4)	25	0	25	25	25	0	25
Bredeney (6)	0	0	0	0	0	0	0

Reports of antibiotic resistance in clinical samples in the ROI confirms that MDR resistance in S. Typhimurium is a growing problem...

With regard to the western world, the emergence of resistance to extended spectrum cephalosporins (antibiotics used in the treatment of complicated *Salmonella* spp. in infants) is cause for concern. Extended spectrum celphalosporin resistance was first identified in *S.* Virchow in Belgium, resulting in a national increase in the prevalence of *S.* Virchow. Approximately one year after detection of this phenotype, this strain was isolated from poultry meat and humans. This strain was also resistant to tetracyclines, sulphonamides, trimethoprim and nalidixic acid in Europe and in the ROI (Table 7). The emergence of resistance to nalidixic acid is also of concern since it is the first step of a two-step mutational resistance to fluoroquinolones, the first choice antibiotics for treatment of *Salmonella* spp. infection in adults.

The emergence of cephalosporin resistance can be seen in many *Salmonella* spp. serotypes in Europe including *S.* Agona, *S.* Derby, *S.* Paratyphi B, *S.* Typhimurium and of particular concern *S.* Infantis. While typically *S.* Infantis is reported to be resistant solely to cephalosporins, resistance is located on a transposon on a self-transmissible plasmid, indicating the possibility of dissemination to other serotypes. In conjunction, cephalosporin resistance is most prevalent in *S.* Infantis in poultry.

Table 7a Antimicrobial susceptibility testing of *Salmonella* spp. in animals in the ROI

N = Number of resistant isolates					
	***S.* Typhimurium**				
Isolates from a monitoring programme	**Cattle (bovine animals)**	**Pigs**	**Gallus (fowl)**	**Turkeys**	**Other animals – Clinical investigations**
Number of isolates available in the laboratory	8	0.6	2	2	0.6
Antimicrobials:	69	58	64	68	66
Tetracyclin	20	0	20	40	20
Florfenicol	30	20	30	60	50
Cefalexin	45	0	100	18	100
Neomycin	33	0	50	50	50
Trimethoprim + sulfonamides	43	43	43	43	0

(Source: Modified from EFSA, 2006a)

In the western world the emergence of resistance to extended spectrum cephalosporins is cause for concern.

Table 7b Antimicrobial susceptibility testing of *Salmonella* spp. in animals in the ROI

N = Number of resistant isolates												
	***S.* Typhimurium**											
	Cattle (bovine animals)		**Pigs**		**Gallus (fowl)**		**Turkeys**		**Other animals – Clinical investigations**		**Other animals – Clinical investigations**	
Isolates out of a monitoring programme	-		-		-		-					
Number of isolates available in the laboratory	268		15		-		0					
Antimicrobials:	N	n	N	n	N	n	N	n	N	n	N	n
Tetracyclines Tetracyclin	220	20	15	12	-	-	0	0	13	3	2	1
Amphenicols Florfenicol	218	25	15	2	-	-	0	0	13	2	3	0
Cephalosporins Cefalexin	41	4	6	0	-	-	-	-	3	2	3	0
Fluoroquinolones Enrofloxcin	221	2	15	0	-	-	-	-	13	0	3	0
Aminoglycosides Streptomycin Neomycin	41	10	7	7	-	-	-	-	3	3	3	0
	169	8	9	4	-	-	-	-	8	1	3	0
Trimethoprim + sulfonamides Trimethoprim + Sulfonamide	223	12	15	10	-		-	-	13	1	3	0
Resistant to 1 anitmicrobial	-	26	-	3	-	-	-	-	-	-	-	1
Resistant to 2 anitmicrobials	-	23	-	2	-	-	-	-	-	-	-	-
Resistant to 3 anitmicrobials	-	7	-	3	-	-	-	-	-	2	-	-
Resistant to 4 antimicrobials	-	1	-	2	-	-	-	-	-	1	-	-
Resistant to > 4 antimicrobials	-	2	-	4	-	-	-	-	-	1	-	-

(Source: Modified from Table P54, EFSA, 2006a)

Antimicrobial resistance in *Salmonella* spp. in Northern Ireland

Much of the recent NI-specific data is consolidated into the Health Promotion Agency Reports (HPA, 2008) which have noted a generalised increase in antibiotic resistance in *Salmonella* spp. isolates from diseased animals, environment and feed-stuffs. However, a recent survey of *Salmonella* spp. isolated from post evisceration pig carcasses and caecal contents, indicated a high incidence of *S.* Typhimurium in caecal contents (19%) and on carcasses (24%) (McDowell *et al.*, 2007). The majority of these were *S.* Typhimurium (DT104) carrying the genomic island SgI1. McDowell *et al.* (2007) reported high rates of resistance to these antibiotics (>50%) but low or no resistance to other clinically important antibiotics, such as, naladixic acid (2.1%), ciprofloxacin (0%) and cefotaxime (0%). The frequency of AMR in human enteric salmonellae in NI is high. However, it is not as high as reported in animal isolates for example, ACSSuT (26- 27%) and nalidixic acid (16%) and much lower for re ciprofloxacin and cefotaxime (< 1%) (Ong *et al.*, 2007). The increased level of resistance to nalidixic acid (caused by single point mutation) is of clinical importance (Stevenson *et al.*, 2007). The AMR profiles of *Salmonella* spp. differ among food animal species. Porcine isolates tend to be resistant to a greater number of antibiotics than bovine or ovine, and turkey isolates are resistant to a greater number of antibiotics than chicken isolates (Table 7). No resistance to third generation cephalosporins or ciprofloxacin have been detected in any animal isolates, with the exception of one case in poultry. This is an important as ciprofloxacin is the drug of choice (DEFRA, 2006). Such intra-species differences may reflect on the intensity of the farming methods, for example, pigs are intensively reared indoors, where illness in one animal is easily spread through the herd. The whole herd may thus be treated, whereas cattle or sheep are field farmed and are treated as individual animals. The age of the infected animal is also important. *Salmonella* spp. from calves for instance, are significantly more resistant to antibiotics than from older bovine animals (EFSA, 2006), although the reasons for this are unclear.

Evidence of food processing impacts

Increased thermotolerance in an MDR strain of *S.* Typhimurium DT104 (in comparison with antibiotic-susceptible strains of *S.* Typhimurium) is of concern. Previous reports have speculated on the mechanisms and implications of *S.* Typhimurium DT104 possessing increased fitness/ survival characteristics, including thermal tolerance, by overcompensation of fitness costs or an increase in *rpoS* expression (EUCAST, 2000; Humphrey, 2001). Walsh *et al.* (2001) reported no difference in heat resistance between antibiotic-susceptible and laboratory-acquired antibiotic resistant (nalidixic acid and streptomycin) strains of *S.* Enteritidis and *S.* Typhimurium at 55°C in chicken meat. However, the same study reported that MDR *S.* Typhimurium DT104 was significantly more heat resistant at 55°C in chicken meat, than the antibiotic-susceptible strains of *S.* Typhimurium and *S.* Enteritidis. A subsequent, larger study by Bacon *et al.* (2003a, 2003b) which examined five MDR and five antibiotic-susceptible strains including *S.* Typhimurium DT104 (at 55°C, 57°C, 59°C and 61°C in tryptic soy broth, with and without glucose), reported no difference in heat resistance between antibiotic-susceptible and MDR *Salmonella* spp. isolates. When antibiotic resistance is induced, some strain variations in

Verocytotoxigenic Escherichia coli (VTEC), in particular O157, are an important cause of gastrointestial illness in the ROI.

acid resistance were observed, but no association between antibiotic susceptibility and the ability to survive low pH was made. Stopforth *et al.* (2008) reported no difference in survival curves between 10 MDR and 10 antibiotic-susceptible isolates of *Salmonella* spp. As yet, there is no conclusive evidence that acquisition of antibiotic resistance confers resistance to food processing stress in *Salmonella* spp. Available data suggests that further experimentation is required.

VTEC – Pathogenic *E. coli* including

Introduction

Verocytotoxigenic *Escherichia coli* (VTEC), in particular O157, are an important cause of gastrointestial illness in the ROI (Epi-insight, 2008). They have emerged as significant pathogens causing a range of severe (10% of patients developing Haemolytic ureamic syndrome (HUS)) and fatal illness. *E. coli* O157:H7 was the first *E. coli* serotype to be associated with this distinctive illness. Additional verocytoxin-producing serogroups, frequently reported in the ROI include, O26, O111, O103 and O145. Infection can be transmitted through food, contaminated water, the environment and by direct contact with animals or humans.

Description of species

E. coli is arguably the best-known and most intensely investigated group of the bacteria found in humans and warm-blooded animals (Park *et al.*, 1999). It was first discovered by Dr. Theodore Eschrich in 1885 (Neill, 1994). *E. coli* are members of the *Enterobacteriaceae* family and are characterised by being facultatively anaerobic Gram-negative rods, motile or non-motile, chemorganotrophic and having both a respiratory and a fermentative type of metabolism. They are oxidase-negative, Vogues-Proskauer negative, methyl red positive, catalase positive, usually citrate negative and have an optimal growth temperature of 37oC (Holt *et al.*, 1994). While *E. coli* are considered to play a role in the maintenance of normal physiological function (Neill, 1994), there are pathogenic strains that cause diarrhoeal disease syndrome (Levine, 1987). Such strains are categorised into the following six groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O:H serogroups. Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Diffuse adhering (DAEC), Enteroaggregative (EaggEC) and Enterohaemorrhagic (EHEC) are different types of *E. coli* strains (Doyle *et al.*, 1997; Sussman, 1997). EHEC are a defined subset of VTEC, which are characterised by the presence of verotoxins (shiga toxins). The term EHEC refers to serotypes of *E. coli* that cause a clinical illness, similar to that caused by *E. coli* O157:H7, which produce one or more phage encoded shiga toxins, possess a 60-megadalton virulence plasmid and produce attaching-effacing lesions in an animal model (Griffin and Tauxe, 2001).

VTEC in food on the island of Ireland

The presence of VTEC in food is of particular concern, as the minimum infectious dose is estimated to be as low as 10 viable cells. Two surveys carried out in the ROI in 2006 and 2007, found none of the retail products tested (238 and 573, respectively) to contain *E. coli* O157 (see Table 8).

Table 8 VTEC in food in the ROI

Food type	Tested for VTEC 2006	Positive 2006	Tested for VTEC 2007	Positive 2007
Fresh meat				
Bovine	9	0	8	0
Pork	2	0	0	0
Poultry	2	0	0	0
Sheep	1	0	1	0
Unspecified meat and Other meat	24	0	0	0
Meat products				
Bovine	28	0	49	0
Pork	65	0	14	0
Poultry	14	0	11	0
Sheep	2	0	0	0
Unspecified meat and Other meat	42	0	11	0
Other foods				
Milk and milk products	8	0	13	0
Fish and fishery Products	2	0	2	0
Fruit and vegetables	5	0	3	0
Juice	0	0	172	0
Soft Drinks	0	0	275	0
Other food	34	0	14	0
Total	**238**	**0**	**573**	**0**

(Source: FSAI, 2009)

The ROI, England, NI, Scotland and Wales have some of the highest reported rates of VTEC infection in Europe.

Other studies carried out on the ROI reported that, 2.4 per cent of beef trimmings, three per cent of beef carcass samples, one per cent of pre- and post-chill lamb carcass samples and 12 per cent of milk filters, were positive for *E. coli* O157. Cagney *et al.* (2004) reported that 2.7 per cent of supermarket mince-meat samples and 3.14 per cent of butcher shops mince-meat samples were positive for *E. coli* O157 (Carney *et al.*, 2006).

Epidemiology of VTEC on the island of Ireland and in Europe

In the ROI there were 115 confirmed and 52[1] probable cases of VTEC notified in 2007 (HPSC, 2007b). The crude incidence rate of 3.9 cases/100,000 population represents a six per cent increase in the number of cases (n=158) reported in 2006. This is the highest annual total of VTEC infections ever reported in the ROI. Young children suffered the highest burden of VTEC with an age-specific rate of 17.5 cases/100,000 population, and all five HUS cases occurred in children aged 1-7 years. Of the 115 confirmed cases, the most common serotype reported was O157 (n=94), followed by O26 (n=13). Two VTEC cases were mixed infections: one VTEC O157 and VTEC O103; the other VTEC O157 and VTEC O113.

In NI, 47 cases of laboratory-confirmed VTEC were reported in 2006 with 17 cases in children aged 0-4 (CDSCNI).

The ROI, England, NI, Scotland and Wales have some of highest reported rates of infection in Europe (European Food Safety Authority, 2005); and in 2006, only Scotland (4.8 per 100,000) had a higher incidence than the ROI. In the EU, 2,905 cases were reported in 2007 with an incidence of 0.6 cases/100,000 population (EFSA, 2009). Data on serogroups was provided by 19 member states[2], and of these over half (54.1%) were serogroup O157.

A survey of Irish beef abattoirs carried out by Whyte (2009) *E.coli* – its prevalence and virulence in Irish beef, sheep and milk. Relay Final Report. Available at: http://www.relayresearch.ie/Public/p_research_project_details.asp?project_id=271 revealed that O157 is the most prevalent of five *E. coli* serotypes capable of causing human illness. No *E. coli* O111 was detected in the survey and despite the fact that O103, O145 and O26 were found only a few of these were pathogenic. *E. coli* O103, O26 and to a lesser extent O157 are quite prevalent in Irish sheep populations. However, only a very small proportion of isolates possess the genes necessary to be highly pathogenic. The risk associated with raw milk appears low. This study highlights the need to establish the virulence in addition to the prevalence of *E. coli* so that more meaningful assessments of risk to public health can be made.

1 The 52 probable cases were associated with a single outbreak

2 Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Luxembourg, Malta, the Netherlands, Poland, Slovakia, Slovenia, Spain, Sweden and the United Kingdom

Antimicrobial resistance in VTEC

To date, antibiotic resistance in VTEC has received little research attention. This is mainly because antibiotic therapy is not recommended in cases of VTEC-associated infection and VTEC-strains have been reported to be slower to acquire resistance than generic *E. coli* strains (Mizan *et al*., 2002; Sanchez *et al*., 2002; Bettelheim *et al*., 2003). However, more recently, multi-resistant strains of VTEC have been isolated from foods (Galland *et al*., 2001; Schroeder *et al*., 2002a; Schroeder *et al*., 2002b, Fitzgerald *et al*., 2003; Schroeder *et al*., 2003), suggesting increasing proliferation of antibiotic resistance among VTEC.

There is currently no surveillance data available on antibiotic resistance in VTEC on the island of Ireland, except for a few published studies in the scientific literature. Walsh *et al*. (2006) compared the antibiotic resistance profiles of *E. coli* O157:H7 isolates (n = 257) recovered from bovine hides, mince-meat (ground beef) and human clinical samples in the ROI to those profiles of a range of Irish non-O157 *E. coli* (O111 and O26) isolates (n=31) from a variety of clinical and veterinary sources (Walsh *et al*., 2006). Four MDR *E. coli* O157:H7 food isolates were identified, with resistance to 10 (one isolate), six (one isolate) and four (two isolates) antibiotics respectively. Another study by Murphy *et al*. (2005) identified 16 antibiotic-susceptible strains of VTEC from Irish bovine milk used in the production of farmhouse cheese. One of these isolates was resistant to streptomycin. Murphy *et al*. (2007) also examined milk supplied (bovines, ovines and caprines) to the Irish farmhouse cheese sector and the manufacture of raw milk ice cream. All of the *E. coli* O157:H7 isolates (n=19) were susceptible to the panel of 15 antimicrobials tested and among the O26 isolates (n=17), three were defined as MDR (ampicillin, tetracycline and streptomycin). The prevalence of MDR is considered to be low in VTEC isolates.

Even less information is available about antibiotic resistance in *E. coli* O157:H7 in NI and the material that is available is usually subsumed within UK wide reports. Some information is available based on veterinary and human clinical cases which suggests that the incidence of antimicrobial resistant *E. coli* O157:H7 remains very low in cattle and sheep isolates, low in pig isolates, and with the exception of tetracycline, very low in human clinical isolates (see Table 9).

There is currently no surveillance data available on antibiotic resistance in VTEC on the island of Ireland...

Table 9 AMR (%) in UK veterinary and human VTEC isolates. (Adapted from AMR, 2004).

Antibiotic	**% of resistant isolates**			
	Cattle	Sheep	Pig	Human
Ampicillin	1	0	8	2
Cefoperazone (cephalosporin)	0	0	0	0
Ciprofloxacin				0
Sulphonamide/trimethoprim	0	0	8	20
Tetracycline	6	0	46	18

Although antimicrobial resistance in *E. coli* O157:H7 is low in most cases, the situation is very different in non-VTEC *E. coli*, where the incidence of resistance to antibiotics is high (>75%) or very high (i.e. >90%) in some animal species, posing the significant risk of transfer of (multiple) resistance to *E. coli* O157:H7 by gene flow through the resistome.

Evidence of food processing impacts

Food processing impacts are of concern when dealing with low infectious dose pathogens, such as, *E. coli* O157:H7, particularly with regard to the processing of ready-to-eat foods, such as, fermented meat. Only one study (Duffy *et al.*, 2006) has compared antibiotic-resistant and antibiotic-sensitive VTEC under food-processing stress. In this study, the impact of laboratory-acquired antibiotic resistance (to nalidixic acid and streptomycin) on the growth and survival of *E. coli* O157:H7 and *E. coli* O26 was examined. The presence of antibiotic resistance did not affect the growth kinetics (lag phases, growth rates) of the VTEC strains, over a 24-hour period at 37°C in laboratory media. The survival of the VTEC antibiotic-resistant strains in orange juice and yoghurt and their D-values at 55ºC were not significantly different to the parent antibiotic-sensitive VTEC strains. However, another study by McGee (2003) reported that acid sensitivity occurred in one out of three antibiotic-resistant (nalidixic acid and streptomycin) *E. coli* O157:H7 isolates. Duffy *et al.* (2006) also reported that the growth kinetics, lag phases and growth rates of an MDR *E. coli* O157:H7 (resistant to 10 antibiotics) over 24-hour at 37°C in laboratory media were

similar to all other strains tested (Duffy *et al*., 2006). However, when subjected to food stresses (acid and heat), this particular MDR *E. coli* O157:H7 isolate, was found to act very differently to the unstressed antibiotic sensitive and antibiotic-resistant VTEC strains. All VTEC strains tested were found to survive for approximately 30 days in orange juice at pH 4.4 and 25 days in yoghurt at pH 4.2. The exception was the MDR *E. coli* O157:H7 isolate which was found to have died off significantly faster ($P<0.05$) in both media, than in the other strains tested. Thermal inactivation studies also showed the MDR strain to be significantly more heat sensitive (D_{55} value = 1.71 min) than all other VTEC strains examined in this study, or indeed in the wider literature (11.13 to 139.2 min) (Juneja *et al*., 1998; Clavero *et al*., 1998; Byrne *et al*., 2002; Huang and Juneja, 2003). Thus, the acquisition of antibiotic resistance does change resistance to other stresses by increasing the AMR host sensitivity to environmental/food processing related stress.

Campylobacter is the most commonly reported cause of bacterial foodborne infection on the island of Ireland.

Campylobacter

Antimicrobial resistance and virulence – *Campylobacter*

Campylobacter is the most commonly reported cause of bacterial foodborne infection on the island of Ireland.

In the ROI in 2006, there were 1,815 notified cases of campylobacteriosis, giving a crude incidence rate of 42.8/100,000 population, a slight increase from 42.5 from the previous year (HPSC, 2006). The highest age-specific incidence rate was in 0-4-year-old children at >120/100,000. Of the 38 per cent of isolates with typing data, 91 per cent were *C. jejuni* and eight per cent were *C. coli*. No data were available on antimicrobial resistance.

In NI in 2006, there were 937 cases of laboratory-confirmed campylobacteriosis and of those 106 were in 0-4-year-old children (CDSCNI).

There were 1,803 notified cases of Campylobacteriosis in 2005 – over five times the number of *Salmonella* spp. cases. Of the infectious diseases caused by members of the *Campylobacter* genus, *Campylobacter* gastroenteritis due to *Campylobacter jejuni* and *Campylobacter coli* is the only form of disease that is of major public health importance (Oncul *et al*., 2003). *Campylobacter* is a naturally transformable microorganism (Wang and Taylor, 1990) that is capable of acquiring a diverse array of Gram-positive (Werner *et al*., 2001) and Gram-negative (Pinto-Alphandary *et al*., 1990) AMR genes. Increasing antimicrobial resistance in both medicine and agriculture in *Campylobacter* is recognised by various national authorities, such as, the Food Safety Authority of Ireland, and global authorities such as the WHO as a major emerging public health concern (Moore *et al*., 2006).

Campylobacter may be transmitted to humans either directly or indirectly. Direct transmission can occur via contact with animals, carcasses or water which has been infected. Indirect transmission can occur through the ingestion of contaminated food or water. In the US, the highest risk factor for campylobateriosis is the consumption of commercially-prepared chicken (Rocourt *et al*., 2003).

In an all island study, *Campylobacter* was isolated most frequently from retail poultry (chicken 49.9%, turkey 37.5% and duck 45.8%) (Whyte *et al*., 2004). However, data from the EU in 2005 showed that the incidence of *Campylobacter* spp. in fresh poultry meat at slaughter ranged very considerably from 4.6 per cent to 56.1 per cent. Similarly, at processing, the incidences ranged from 3.8 per cent to 51.9 per cent and at retail between 3.1 per cent and 66.4 per cent. Livestock production, in particular poultry production, has become very intensive across the island of Ireland during the last 20-30 years. Research has shown that by the third or fourth week of production, most poultry flocks are contaminated to some extent with *Campylobacter* spp., resulting in the eventual spread of the organism to almost all members of the flock. In the above all Ireland study (Whyte *et al*., 2004), *Campylobacter* was also isolated from beef (3.2%), pork (5.1%) and lamb (11.8%) and fresh mushrooms (0.9%). A number of more recent UK/NI/EU studies have reported *Campylobacter* isolation rates of 63 per cent (DEFRA, 2006), 98.3% (EFSA, 2010) and 86.2 per cent (Meldrum and Wilson, 2007) from poultry.

Recent isolation rates include raw meat (4.8%), pork (2%), lamb (8.5%) and raw milk (1.9%). Madden *et al.* (2007) noted 24.8 per cent incidence in NI beef cattle faeces, suggesting a potential source for carcass contamination, although the incidence of *Campylobacter* on NI beef cattle carcasses is very low (Madden *et al.*, 2001).

AMR is a common feature of *Campylobacter* isolated from food of animal and non-animal origin. In a European study in 2006, *Campylobacter* isolates from poultry meat were found to have a high level of resistance to ciprofloxacin (30.6%) (Piddock, 2006). Resistance to this antibiotic was also high in isolates from fowl (Gallus gallus), pigs, cattle, at levels of 31.6 per cent to 56.7 per cent. In addition, resistance to tetracycline was found to be very common (EFSA, 2006). In food animals, the prevalence of resistance to erythromycin is generally higher in *C. coli*, in particular in *C. coli* isolates from pigs, than in *C. jejuni* (Engberg *et al.*, 2001). In Spain, it was found that the rates of erythromycin and quinolone resistance in *C. coli* from pigs were 81 per cent and 100 per cent, respectively (Saenz *et al.*, 2000). Antibiotics used in high concentrations in animal feed can be found in animal manure, which can end up as plant fertiliser (Kühn *et al.*, 2003). In the US, poultry litter is spread on fields as a fertiliser which can be a source of MDR bacteria (Gangle, 2005). This has been shown in vitro by Batchelder and colleagues in 1981 (Batchelder, 1982).

Information on the AMR profiles of *Campylobacter* spp isolated from animal/food sources in NI/UK is generally regarded as weak, with the last UK survey of AMR in *Campylobacter* spp isolated from animals (carried out on pigs only) in 2003. Although, there appears to be a trend of increasing resistance to quinolones (ciprofloxacin) and macrolides (erythromycin, Table 10), there is little updated data available to confirm this suggestion (Rao *et al.*, 2005). The majority of data originates from poultry isolates, and as such may not be representative of all food animal isolates. It has been reported that a high proportion of animal *Campylobacter* spp are resistant to ampicillin/tetracycline (>40%) and ciprofloxacin/nalidixic acid (>20%). A recent report stated that 22 per cent of chicken isolates have multiple AMR (resistant to three antibiotics or more), a significant advance from the report of Randall *et al.* (2003) which suggested that 3.8 per cent of *Campylobacter* spp from combined human and animal origins were multi-resistant. It is not clear if this increased MAMR is matched in other food species, especially as wide variations have been reported in the AMR profiles of *Campylobacter* isolates from different types of retail meats. In general, *Campylobacter* spp isolated from pork and poultry have higher and broader resistance to antibiotics than lamb or beef. This may be attributable to the intensive farming methods used in pig and poultry rearing, where mass medication is relatively common and high stocking levels may facilitate dissemination of pathogens and AMR genes between animals (Turnidge, 2004; McGill *et al.*, 2006).

A high proportion of animal *Campylobacter* spp are resistant to ampicillin/ tetracycline and ciprofloxacin/nalidixic acid.

On the island of Ireland, a high degree of similarity between the AMR/MAMR profiles of clinical and food *Campylobacter* spp isolates has been reported (McGill *et al.*, 2006), along with increased resistance to clinically relevant antibiotics, such as, ciprofloxacin, erythromycin and tetracycline among food isolates. In NI, Rao *et al.* (2005) reported concurrent increases in erythromycin resistance in *C. jejuni* isolates from humans and from poultry during the period 1999-2003, although susceptibility to other antibiotics (penicillin, cefalexin, chloramphenicol, ciprofloxacin and tetracycline) remained static over this period. The above noted increase in erythromycin resistance is of clinical significance.

The above data suggests that *Campylobacter* spp isolated from animal/food and those isolated from human campylobacteriosis may be linked via the food chain (Rao *et al.*, 2005, McGill *et al.*, 2006), although GE *et al.* (2002) noted significant delays between peaks of isolation. There is, however, a reported lack of temporal association between human infections and contamination in retail chicken products, particularly during peak seasons (Wilson, 2002). This may simply be due to the incubation period for campylobacteriosis infection of up to 10 days combined with the time taken for isolation, identification and reporting of the condition to the relevant public health authorities, which can be up to two weeks. The data made available in the report by Wilson (2002) indicates that a peak in *Campylobacter* spp. isolation from raw chicken is often followed 4-5 weeks later by a peak in isolation from humans.

Table 10 AMR profiles of Campylobacter spp in NI.

	% isolates resistant				
	Clinical 2001/2* (*C. jejuni*)	Poultry meat 2001/2* (*C. jejuni*)	Other retail food products 2001/2* (*C. jejuni*)	Pig meat 2002** (*campy spp*)	Poultry meat 2004** (*C. jejuni*)
Amp	17	24	32	21	64
CHl	4.1	7.8	9	0	5
Cip	13	18	14	21	21
Ery	6.2	5.2	18	55	3
Nal	17	18	0	25	23
Tet	10	12	18	69	41

Data from
** (McGill et al. 2006)*
***AMR report 2004*

There are examples from many countries where fluoroquinolone-resistance rates are similar in isolates from poultry products and humans...

Fortunately, most cases of human *Campylobacter enteritis* do not require antimicrobial treatment, as the symptoms are usually clinically mild, and self-limiting in healthy adults (Casburn-Jones and Farthing, 2004). A survey conducted in the ROI in 2003 found that only 7.4 per cent of those with acute gastroenteritis reported were taking antibiotics (Igoe *et al*., 2003). However the symptoms of campylobacteriosis can vary from mild self-limiting enterocolitis lasting 24 hours to more severe illness including diarrhoea, abdominal cramps and vomiting which can last up to 10 days. For those immunosuppressed or with chronic intestinal disorders, *Campylobacter* infection can have more serious consequences and long-term antibiotic therapy may be necessary (McGill *et al*., 2006). The use of Quinolones (ciprofloxacin) is considered to be the first choice for treatment as they are also considered useful for the prophylaxis of travellers' diarrhoea (Ericsson, 2003; Piddock, 2006). This approach was adopted because the symptoms of *Campylobacter enteritis* essentially mimic those of bacterial gastroenteritis caused by other enteric pathogens, such as, *Salmonella* spp. and *Shigella* spp. Furthermore, the susceptibility of these pathogens to fluoroquinolones, empirical treatment for severe gastroenteritis with these drugs was used before culture tests were confirmed (Alfredson and Korolik, 2007). In 1991, Endtz *et al.* reported quinolone resistant *C. jejuni* and *C. coli* isolated from humans were emerging in The Netherlands (Endtz *et al*., 2002). Patients infected with fluoroquinolone resistant *C. jejuni* tend to have longer duration of diarrhoea than patients with fluoroquinolone-sensitive isolates (Phillips *et al*., 2004). Thus, macrolides (erythromycin) became the most commonly used agent for treatment of *C. enteritis* (Engberg *et al*., 2001).

It is interesting to note that the resistance of *C. jejuni* and *C. coli* isolates from humans in The Netherlands coincided with the introduction of fluoroquinolones in veterinary medicine in a number of countries (Aarestrup *et al*., 1998; specifically in poultry (Endtz *et al*., 2002). Recognition of this relationship led to bans in the veterinary use of such products, for example, sarafloxacin was withdrawn from use during 2001 (Gupta *et al*., 2004), and in 2005, the Food and Drug Association banned the use of enrofloxacin, previously used in the treatment of colibacillosis in chickens and turkeys (Higgins *et al*., 2007).

There are examples from many countries where fluoroquinolone-resistance rates are similar in isolates from poultry products and humans (Endtz *et al*., 1990; Wegener, 1999; Saenz *et al*., 2000). In the UK, enrofloxacin was first licensed in late 1993. Previously, domestically-bred chickens were less frequently infected with quinolone-resistant *Campylobacter* than imported chicken products. Researchers found a correlation between the percentage of human isolates with antibiotics resistance and estimates of the amount of imported chicken consumed in the UK (Payne *et al.* 2002). In recent data from Spain and Taiwan, rates of erythromycin resistance were 17 per cent for both countries in *C. jejuni* isolated from foods, whereas for *C. coli* the rates were 50 per cent and 83 per cent, respectively (Li *et al*., 1998; Saenz *et al*., 2000). Macrolides, the current treatment of choice for campylobacteriosis, have been banned for use as a growth promoter in the EU since July 1999 (Casewell *et al*., 2003). However, the high incidence of resistance to erythromycin in *C. coli* isolates from pigs has been related to extensive veterinary use of macrolides (Gibreel and Taylor, 2006; Kim *et al*., 2006) during pig production.

Evidence of food processing impacts

Since consumption of poultry is one of the major risk factors in acquiring campylobacteriosis it is worth considering the impact of poultry processing on antibiotic resistance of this pathogen. Slaughter and processing provide opportunities for reducing *C. jejuni* counts on food-animal carcasses. Bacterial counts on carcasses can increase during slaughter and processing steps. In one study, up to a 1,000-fold increase in bacterial counts on carcasses was reported during transportation to slaughter (Stern *et al.*, 1995). Research studies carried out on chickens (Izat *et al.*, 1988) and turkeys (Acuff *et al.*, 1986) at slaughter, found that bacterial counts increased by approximately 10-to-100 fold during defeathering with the highest level found after evisceration. However, bacterial counts on carcasses declined during other slaughter and processing steps. For instance, Oosterom *et al.* (1983) reported that forced-air chilling of swine carcasses caused a 100-fold reduction in carcass contamination. In Texas, in turkey plants, scalding reduced carcass counts to near or below detectable levels (Acuff *et al.*, 1986) and the addition of sodium chloride or trisodium phosphate to the chilled water in the presence of an electrical current reduced *C. jejuni* contamination of chilled water by two $\log_{10}$ units (Li *et al.*, 1995). In a slaughter plant in England, the use of chlorinated sprays and the maintenance of clean working surfaces resulted in a 10-to-100 fold decrease in carcass contamination (Mead *et al.*, 1995). In another study, lactic acid spraying of swine carcasses reduced counts by at least 50 per cent to undetectable levels (Epling *et al.*, 1993). Furthermore, a radiation dose of 2.5 KGy reduced *C. jejuni* levels in retail poultry by 10 log10 units (Patterson, 1995). Freezing or chilling of poultry meat has been shown to greatly reduce the number of live *Campylobacter* present on the product. In addition, it is thought that the aeration resulting from preparation of ground meat products helps reduce the number of viable cells of *Campylobacter*, as this organism is sensitive to high oxygen tension.

During the slaughter of cattle and swine, fresh carcasses are cooled by forced air ventilation. This treatment both temporarily freezes and dries the surface, and this process has been documented to effectively reduce the number of *Campylobacter* cells on the surface of the carcass (Chang *et al.*, 2003). Red or white meat undergoing any heat treatment or freezing during processing will harbour less *Campylobacter* than meat produced without such treatment. Furthermore, meat which is dried, cured, salted, smoked, irradiated or exposed to other preservation methods, will also harbour less *Campylobacter* compared to the unpreserved product. The capacity of different treatment processes to affect the microbiological status of meat products is an area of intense interest. However, public demand for fresh poultry meat (e.g. cuts of breast meat), over whole frozen carcasses has contributed significantly to the decade-long increase in the incidence of human *Campylobacter* observed in many industrialised countries.

...when resistance emerges in Campylobacter in animals, resistant Campylobacter can be transmitted to humans.

There are no significant biological reasons why resistant *Campylobacter* should not transmit equally well from animals to humans, as does sensitive *Campylobacter.* Hypothetically, there may be a small fitness loss when a strain of *Campylobacter* mutates and becomes resistant to quinolones, however, this loss of fitness may be compensated in successive generations. Thus, when resistance emerges in *Campylobacter* in animals, resistant *Campylobacter* can be transmitted to humans. In addition, results of a recent study have indicated that certain strains gain increased fitness when acquiring fluoroquinolone resistance mutations (Luo *et al*., 2005). This process is undesirable as it suggests that the acquisition of AMR can lead indirectly to the emergence of more widely generally, competitive AMR strains. Doyle and Erickson (2006) noted such effects as examples of the complex nature of antibiotic resistance and the large data gaps that exist in making informed scientific decisions on the use of antimicrobials in animals used as food.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Emerging/new pathogenic bacterial hazards

Staphylococcal food poisoning occurs after the ingestion of food contaminated with the staphylococcal enterotoxin(s) (A, B, C1, C2, C3, D, E, G, H, I, J, K, L, M, N and O) (Smyth *et al*., 2006). It is mainly caused by human strains of S. aureus producing SE(A) and/or SE(D), with the majority of strains producing SE(A) alone. The amount of toxin necessary to cause illness depends on the susceptibility of the person. However, epidemiological studies have shown that only one µg of SE toxin can cause food poisoning. It should be noted that, to produce this amount of SE, an entertoxigenic strain needs to grow to levels of 105 to 106 cells per gram or ml. The onset of food poisoning symptoms usually occurs between one and seven hours after the ingestion of food containing SE. Symptoms include nausea, vomiting, abdominal cramps and diarrhoea. In severe cases, fainting may occur. Recovery is usually rapid, i.e. within two days.

Outbreaks and sporadic cases of staphylococcal food poisoning have been linked with foods such as, cheese, salami, bakery products, pasta, canned meat, canned fish and canned vegetable products. In relation to cheese, failure of the starter culture may provide an opportunity for *S. aureus* to grow and produce SE. Staphylococcal food-borne disease has been reported as the second most common cause of reported foodborne illness in the US (Halpin-Dohnalek and Marth, 1989). In the EU, 164 outbreaks were attributed to *Staphylococcus* spp. in 2005 (European Food Safety Authority, 2006). This represented 3.1 per cent of all outbreaks reported. In 2004, only three cases of staphylococcal food poisoning were reported, in 2005 there were six cases and in both 2006 and 2007, no cases were reported in the ROI, however it is thought that this illness is under-report because the symptoms are generally not severe enough for the patient to visit their General Practitioners (Health Protection Surveillance Centre, 2004). Furthermore, because staphylococcal food poisoning is toxin mediated and generally self-limited, antibiotics are not used for the treatment of this illness (Gill and Hamer, 2001).

MRSA is generally regarded as a health-care-acquired, or increasingly, a community-acquired pathogen. Notably, MRSA was not reported as a cause of outbreaks of gastroenteritis until 2002 in the US. The reason is that many *S. aureus* isolates obtained as part of outbreak investigations may not be tested for antibiotic susceptibility – and, therefore, Methicillin-Resistant Strains (MRS) may go unrecognised as the cause of foodborne outbreaks of acute gastroenteritis. MRSA are as likely to produce enterotoxins as are methicillin-sensitive strains (Jones *et al*., 2002).

...infection of humans by transmission through food products contaminated with animal MRSA may occur.

In 2002, a reported outbreak in the US, found that MRSA contaminated food was the vehicle in a cluster of illnesses affecting low-risk persons within the community. This food was probably contaminated by an asymptomatic carrier whose only apparent exposures were intermittent visits to a nursing home (Jones *et al*., 2002). This outbreak could be an example of second-generation spread of a health-care-associated pathogen into the community. While antibiotic-resistant strains are not expected to be clinically more virulent or challenging in the setting of acute outbreaks of gastroenteritis, MRSA may cause soft-tissue and other infections in the community that are difficult to treat.

In the 1990's an MRSA outbreak causing 21 cases of septicaemia and five deaths occurred in the University Hospital Rotterdam, in The Netherlands. The initial reservoir for the outbreak strain was probably a dietary worker, who carried MRSA in his throat and prepared food for patients on the haematology unit. Transmission of MRSA by contaminated food had not been described before this outbreak (Kluytmans *et al*., 1995).

A study carried out in Korea from May 2001 to April 2003, found that, out of a total of 1,913 specimens from cattle, pigs, and chickens, 421 specimens contained S. *aureus*. Of the 421 specimens, 15 were *mecA*-positive MRSA isolates; twelve were obtained from dairy cows and three from chickens. The genomes of the six animal MRSA isolates, were very closely related to those of some human MRSA isolates and were a possible source of human infections caused by consuming contaminated food products made from these animals (Lee, 2003). These results suggested that infection of humans by transmission through food products contaminated with animal MRSA may occur. More recently, a number of reports have reflected increasing concerns in this area (Kitai *et al*., 2004; Lee *et al*., 2003) especially considering ongoing increases in resistance to antibiotics which are typically part of MRSA's resistant profile. Within the last year, MRSA has been reported in pigs, chickens and cattle in a number of European countries, but this organism is not yet included in UK/ROI food surveillance reports.

While *Staph aureus* food poisoning is toxin-mediated and so the impact of MRSA food poisoning may not be more severe than susceptible SA, the use of antimicrobials in food animals poses a threat to public health through the introduction of new sources of MRSA into the human population. In The Netherlands, it has been demonstrated that pig farmers have a 760-fold increase in carriage of MRSA over the general population, and there is evidence that the source of the MRSA is the pigs which have been exposed to antimicrobials. Hence, pig farmers are at a greater risk of exposing MRSA to their contacts, which in turn may affect the wider community. This source of MRSA threatens the highly successful efforts in The Netherlands to control MRSA in healthcare facilities.

Clostridium difficile

Clostridium difficile is not currently considered a foodborne pathogen and is usually found in the human large intestine (bowel). It is the most common cause of diarrhoea following antibiotic therapy and almost all patients who develop *C. difficile* diarrhoea are taking, or have recently been prescribed antibiotic therapy. A small proportion (less than one in 20) of the healthy adult population carry a small amount of *C. difficile*, but it is kept in check by the normal bacterial population of the intestine. *C. difficile* can also form spores which allow it to survive in the environment outside the body. These spores protect it against heat and chemical disinfectants. Most infections are reported from hospitals and nursing homes, but can also occur in the community.

In most cases, *C. difficile* causes a relatively mild illness. However, infection may result in serious illness and even fatalities in patients of advanced age, and/or suffering from a serious underlying illness, or with conditions that compromise the immune system (e.g. cancer). In addition, patients who have recently had gastrointestinal surgery or those who have spent a long time in hospital or other healthcare setting are also at risk.

C. difficile infection is generally not associated with individuals whose immune system is not already compromised. People can become infected if they touch items or surfaces that are contaminated with faeces and then touch their mouth or mucous membranes. Healthcare workers can spread the bacteria to other patients or contaminate surfaces through hand contact.

The ability of *C. difficile* to form spores allows it to survive for long periods in the environment (including areas around the patient's bed, the toilet areas, sluices, commodes, bed pan washers, on floors etc). Apart from the above principal symptom (diarrhoea), *C. difficile* infection is also associated with fever, loss of appetite, nausea and abdominal pain/tenderness. Generally, *C. difficile* takes advantage of the disruption of the normal, healthy intestinal bacteria by antimicrobial therapy. When *C. difficile* is not challenged by the normal bacterial gut flora, it multiplies in the gut and produces toxins that damage the cells lining the intestine, resulting in violent and profuse diarrhoea.

The presence of these organisms is normally detected by an Enzyme-linked Immunosorbant Assay (ELISA) assay binding *C. difficile* toxin, a process that does not differentiate among the 100 strains of *C. difficile* which have so far been identified. Recently, *C. difficile* Type O27 (first identified in the UK in 1999) has been demonstrated as the predominant strain in two outbreaks in the UK in 2004-2005. This strain was previously associated with a large severe outbreak in hospitals in Canada (Quebec) and North-Eastern USA commencing in 2000. Cases of infection with strain type O27 have been reported in other European countries, including the ROI (Epi-insight, 2006a). Early reports suggest that this strain is more virulent, since it produces a higher amount of toxin, than other ribotypes examined to-date. The production of high amounts of toxin in this ribotype, is the result of a deletion in the gene which normally restricts toxin production. *C. difficile* Type O27 is believed to cause a greater proportion of severe disease,

...published data comparing human and animal isolates revealed the presence of C. difficile in food.

and to have a higher rate of mortality. This is not helped by the ease of transfer of *C. difficile* O27 and infection between patients or its increasing resistance to fluoroquinolones. Resistance to fluoroquinolones and administration of these antibiotics has emerged as an important risk factor for *C. difficile* associated diarrhoea in an epidemic in Quebec.

Recently published data comparing human and animal isolates revealed the presence of *C. difficile* in food. This finding strongly suggests that transmission from food animal reservoirs is a possible source for community-associated infections (Rupnik, 2007). *C. difficile*-associated disease or asymptomatic carriage has been described in animal species (Songer and Anderson, 2006), but the *C. difficile* types in the human and the animal populations have not been compared in detail. If animals are indeed a potential source of *C. difficile* infection, food could be one of the transmission routes from animals to humans. Approximately 20 per cent of retail ground meat samples or other retail meat products have been shown to contain *C. difficile* (Rodriguez-Palacios *et al.*, 2007) and at least some of the ribotypes found in meat (O77, O14, M26, M31) have been recovered from dogs, calves and humans. Type M26 is identical in a number of molecular characteristics (toxinotype III, 18-bp deletion in *tcdC*, presence of binary toxin) to type O27, and was also found to be resistant to levofloxacin and clindamycin. However, the ribotyping and pulsed-field gel electrophoresis profiles of Type M23 are only 80 per cent similar to O27 strains from humans. *C. difficile* could contaminate meat during processing, but another possibility is that spores are already present in the muscle tissue. The latter possibility has been described for other clostridial species in horses, but not for *C. difficile* (Vengust *et al.*, 2003).

Whether antibiotic resistant strains of *C. difficile* are affected differently by processing compared to non-antibiotic-resistant strains remains to be determined, but further evaluation is still required on the exact role of food as a route of transmission of this micro-organism.

A marked increase in *Clostridium difficile* Associated Disease (CDAD) has been reported across Europe and North America over the past decade or so, and for the EU alone, the potential cost of CDAD has been estimated as three billion euro annually (Kuijper *et al.*, 2006). Kuijper *et al.* (2006) noted that CDAD is an increasing threat to human health, with multiple issues of concern, including an increase in the elderly and vulnerable population in Europe, and an increase in reports of resistant and more virulent strains of *C. difficile* and *C. difficile* strains that are more difficult eliminate from the environment due to resistance to many cleaning agents (Kuijper *et al.*, 2006). The control of *C. difficile* is an important challenge in human healthcare and it is essential that no new source from animal reservoirs is allowed to occur.

Commensal non-pathogenic bacterial hazards

Commensals may play a role in the spread of antibiotic resistance, through the dissemination of resistance genes to susceptible bacteria. In humans and animals this is most likely to occur in the intestinal tract, where the ability of bacteria to colonize, transfer and accept resistance determinants remains important. A knowledge gap exists on the prevalence of antibiotic resistance genes in the animal and human intestines. The two most studied bacteria of the gastrointestinal tract (*Enterococci* and *E. coli*) make up only one per cent of the total intestinal microflora. The majority of intestinal bacteria cannot be cultivated, and much of the limited knowledge that exists, is about the minority, cultivatable (potentially atypical) populations (EFSA, 2008).

The application of culture-free and genomically-based forms of detection and differentiation to these complex populations is likely to make considerable contributions to our understanding of the interactions of such commensals with enteric pathogens. This in turn should clarify the relative significance of the presence of antibiotic genes in commensals in the acquisition and dissemination of these genes in zoonotic/enteric pathogens.

However, it is also important to note that many of the commensals which are amenable to current analytical methods have already been shown to carry qualitatively and quantitatively significant complements of antibiotic-resistant genes. For example, in recent times there have been significant increases in the percentage of vancomycin-resistant *Enterococcus faecium* (37.1%) and ciprofloxacin-resistant *E. coli* in the ROI, while the proportion of MRSA has remained stable, albeit high, at approximately 42 per cent for the past four years (Epi-Insight, 2006b).

Enterococcus spp

Enterococci are Gram positive, facultative bacteria with low intrinsic virulence. They are natural inhabitants of humans and animals and are occasional opportunistic pathogens of the urinary tract, bloodstream, intra-abdominal and pelvic regions, surgical sites and the central nervous system (Murray and Weinstock, 1999). *Enterococcus faecalis* are responsible for 80-90 per cent of human enterococcal infections (Jones *et al.*, 2004) and *Enterococcus faecium* is generally responsible for the remainder. Both species are reported to be intrinsically resistant to cephalosporins, low concentrations of aminoglycosides, clindamycin, fluoroquinolones and trimethoprim-sulfamethoxazole. In addition, many strains harbour transmissible genetic elements for acquired resistance to tetracycline, erythromycin and chloramphenicol and most importantly glycopeptide antibiotics (vancomycin, teicoplanin). The mechanism of vancomycin and teicoplanin resistance in *Enterococci* is based on the presence of a particular type of peptidoglycan precursor terminating in D-Ala-D-lactate instead of D-Ala-D-Ala, the normal binding site of glycopeptides (Bugg *et al.*, 1991). The key enzymes involved are specific ligases VanA or the less common ligase VanB. The gene cluster coding for resistance contains four or five other genes in addition to the ligase. The additional genes which contribute to the phenotype include; *vanR, vanS, vanH, vanX or vanZ*. This gene cluster is often associated

In 1996, there were 265 reports of *E. faeclum* bacteraemia in the ROI, of which 37.1 per cent were vancomycin resistant.

with a transposon (Tn1546), highlighting the potential for resistance transfer (Arthur and Courvalin, 1993). Other clinically important resistance types include *vanC, vanD* and *vanE* type ligases (Cetinkaya *et al*., 2000). During the past two decades, the incidence of hospital-acquired infections has risen and these are increasingly associated with MDR (Linden *et al*., 2007). While vancomycin resistance may compromise the treatment of nosocomial infections, there is concern for the eventual transfer of vancomycin resistance to MRSA and the serious consequences that this might entail.

Antimicrobial resistance in *Enterococcus faecalis*

In 1996, there were 294 reports of *E. faecalis* bacteraemia in the ROI, of which 3.7 per cent were vancomycin–resistant isolates. While this percentage may seem low, the ROI has one of the highest proportions of vancomycin–resistant *E. faecalis* in Europe (Epi-Insight, 2007b)

Antimicrobial resistance in *Enterococcus faecium*

In 1996, there were also 265 reports of *E. faecium* bacteraemia, of which 37.1 per cent were vancomycin resistant, the proportion of vancomycin resistant isolates had increased by 6.4 per cent per year. Of the 265 strains isolated, 25.6 per cent were found to be MDR, showing an increase of six per cent per year, reported between 1991 and 1996 (Epi-insight, 2007b).

Foods associated with *Enterococci*

Enterococci are widely distributed in the environment, human and animal gastrointestinal tracts, and are frequently isolated from raw milk, meat, vegetables, or any food that comes in contact with soil or surface water. However, their presence in food products is not always correlated with faecal contamination, making them inefficient hygiene indicators in food processing plants (Birollo *et al*., 2001). The ability of this species to survive pasteurisation temperatures and grow under a wide range of adverse conditions (high and low temperatures, pH and salinity) enables them to persist in processed food products. *Enterococci* are also associated with fermented foods, (such as, cheeses and meats) and are widely used as starter cultures in the food industry.

Evidence of food processing impacts

There is currently no literature available on any differential effects of food processing stresses on antibiotic-susceptible and antibiotic-resistant *Enterococci*. However, there is evidence that conditions within the food-chain may assist in the dissemination of resistant genes, for example, there are reports that the *vanA* gene has been transferred *in vivo* from chicken isolates to human strains (Lester *et al*., 2006). This worrying report highlights the real potential for the transfer of antibiotic resistance from animal/food isolates to human isolates within the food chain, and underlines the need for further investigation of the factors controlling or stimulating such transfers, especially when one considers the types and incidences of AMR involved.

Non-pathogenic *E. coli*

Description of Species

Previously described see pathogenic *E. coli* including VTEC (Section 4).

Antimicrobial resistance in *E. coli*

In the ROI in 2006, 21.5 per cent of *E. coli* were resistant to drug ciprofloxacin, a fluroquinolone drug. This has increased significantly from 5.4 per cent in 2002, at a rate of approximately four per cent increase per year. In wider terms, *E. coli* have also been found to be resistant to ampicillin (70.7%), third generation cephalosporin's (4.2%), aminogycosides (7.7%) and Extended Spectrum ß-Lactamases (ESBL) (20.8%). The above levels of fluoroquinolone-resistance incidence are higher than in the rest of Europe, while the levels of cephalosporin and aminoglycoside resistance are lower. On a positive note, no significant increase in ESBL-producing bacteria has been observed in the ROI in recent years. Overall, nine per cent of *E. coli* tested in 2006 were reported to be MDR resistant. This is in contrast to 2.4 per cent in 2002, an increase of approximately 1.7 per cent per year (Epi-Insight, 2007b).

Food associated with *E. coli*

Food products particularly associated with outbreaks of *E. coli* food poisoning include; raw ground meat, fermented meat, raw unwashed fruits and vegetables (in particular spinach and sprouts), raw milk, unpasteurised juice,or foods contaminated by infected food workers via faecal-oral route. (For further information see verocytotoxigenic *E. coli*).

Evidence of food processing impacts

Evidence of the impact of food processing on antibiotic resistant *E. coli* has been previously discussed in the section on non-pathogenic VTEC. No information is available on the impact of food processing stresses on ABR in commensal *E. coli*.

Probiotics added to food

Bacteria have been added to foods for a long time, and their application in the production, flavour and texture modification of a diverse range of food items remains a very important activity of the food industry. For example, bacterially-cultured dairy products remain the driving force in the growth of dairy food consumption with generated retail sales close to $4.8 million (€3.8 million[3]; £3.4 million) in 2005 (Cogan *et al*., 2007). Lactic acid bacteria (LAB) play a vital role in the manufacturing, flavour, texture and the development of fermented dairy foods. These include lactobacilli, lactococci, streptococci, enterococci, pediococci and leuconostocs. More recently the health benefits of these bacteria have been studied, with LAB such as, lactobacilli, enterococci or *Bifidobacteria* spp. being used in the production of probiotic products.

Most food-associated LAB have 'Generally Regarded As Safe' (GRAS) status, since there have been no reports of clinical cases associated

3 Currency exchange rate = $1USD is equivalent to 0.7920 euro and 0.7016 pound sterling (accessed 17th February, 2009)

with industrial starter strains. A few clinical cases associated with probiotic *Lactobacillus rhamnosus* have been reported (Rautio *et al*., 1999; de Groote *et al*., 2005), however these incidences are quite isolated given the extensive use of LAB. This suggests that the chances of bacterial infection associated with LAB are remote, and that the presence of antibiotic-resistance genes in LAB does not present any significant, direct challenges to clinical medicine. However, there are now increasing concerns that antibiotic resistant genes present in non-pathogenic bacteria (like LAB) can be transferred to pathogenic strains. The fact is that many fermented dairy products and meats that are not heat-treated before consumption may provide a vehicle for antibiotic-resistant bacteria with a direct link between the animal indigenous microflora and the human gastrointestinal tract (Mathur and Singh, 2005).

Few comprehensive studies exist on antibiotic resistance in LAB of food origin, in particular lactococci and lactobacilli. Research has mainly focused on the opportunistic pathogen *Enterococci*. Since *Enterococci* have been previously discussed in this report, current antibiotic-resistant genes are listed for non-enterococcal LAB and *Bifidobacteria* spp. (Table 11).

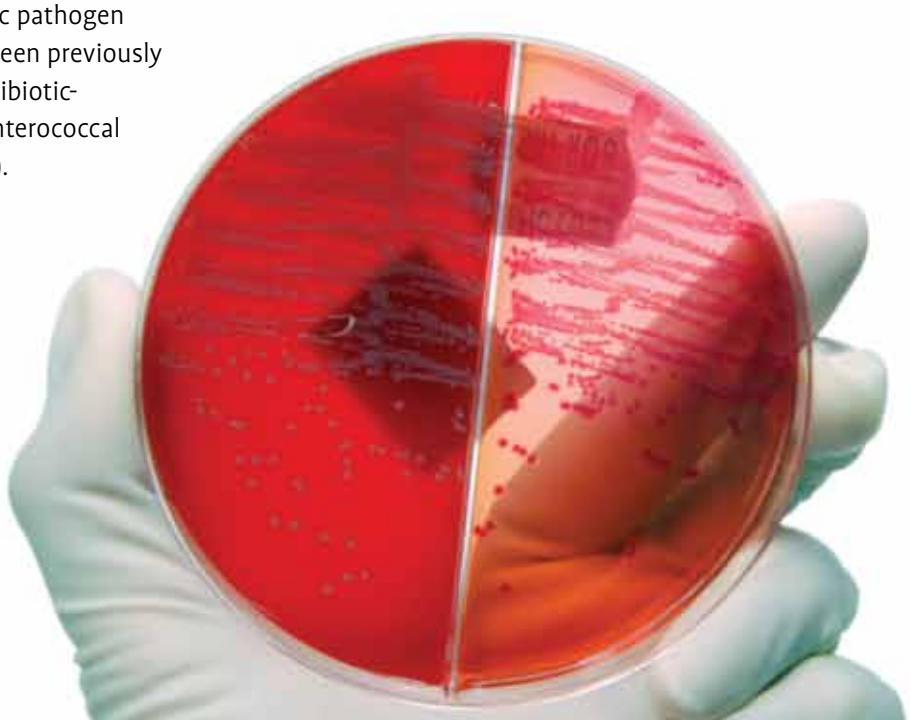


Table 11 AMR genes in non-enterococcal LAB and *Bifidobacteria* spp. (Modified from Ammor et al., 2007)

Species/Strain name	Resistance Gene(s)	Strain Origin
Bifidobacterium longum	tet(W)	Animal, Human
	tet(M)	Human
Bifidobacterium spp.	tet(W)	Animal, Human, Probiotics
Lactobacillus acidophilus	aaa(6')Ie-aph(2")Ia	Animal
	tet(M), erm(B)	Dairy produce, Human
Lactobacillus alimentarius	tet(M)	Fermented Dry sausage
Lactobacillus animalis	erm(B)	Animal
Lactobacillus casei	tet(M), erm(B)	Dairy produce, Human
Lactobacillus crispatus	erm(B)	Animal, Human
	tet(M)	Human
Lactobacillus curvatus	tet(M)	Fermented dry sausage
Lactobacillus fermentarium	tet(M), erm(B)	Human
	erm(LF), vat(E-1), tet(M)	Dairy produce
	erm(B)	Animal
Lactobacillus gasseri	tet(M), erm(B)	Dairy produce, Human
Lactobacillus johnsonnii	erm(B)	Animal, Human
	tet(M)	Human
Lactobacillus plantarum	tet(M), erm(B)	Dairy produce, Fermented dry sausage, Human
	tet(S)	Human
	cat-TC	Pork
Lactobacillus reuteri	tet(W), Inu(A)	Human
	erm(B), erm(T), cat-TC	Animal
Lactobacillus rhamnosus	tet(M), erm(B)	Human

A number of AMR genes are found in non-enterococcal LAB and Bifidobacteria spp.

Lactobacillus sakei	tet(M)	Fermented dry sausage
Lactobacillus salivarius	aaa(6')-aph(2"), erm(B)	Animal
Lactobacillus spp.	erm(T)	Animal
	tet(M), tet(O), tet(T)	Human
	tet(K), tet(S), tet(W), tet(36)	Human
Lactobacillus lactis	tet(M), tet(S), mdt(A), cat, str	Dairy produce
	erm(T)	Animal
Leuconostoc citreum	tet(S)	Sausage Process line
Pediococcus acidilactici	erm(B)	Animal, Dairy produce
	aaa(6')-aph(2")	Animal

Resistance genes: *aac*, aminoglycoside acetyltransferase; *ant*, aminoglycoside adenylyltransferase; *aph*, aminoglycoside phosphotransferases; *cat* chloramphenicol acetyltransferase; *erm*, erythromycin resistant gene; *Inu*, lincosamide resistance gene; *mdt*, multiple drug transporter; *str*, streptomycin resistant gene; *tet*, tetracycline resistant gene; *vat*, streptogramin A acetyltransferase.

Conjugative plasmids for lactose utilization (and/ or proteinase activity) were first reported in *Lactococcus lactis* (Fitzgerald and Gasson, 1988). Since then, many R-plasmids have been reported in *Lactobacilli* (*reuteri, fermentum, acidophilus, plantarum*) and *Lactococcus* (*lactis*) (Lin *et al*., 1996; Fons *et al*., 1997; Vescovo *et al*., 1982, Danielsen, 2002), but the rates of plasmid transfer from these bacteria has been reported to be low (Mathur and Singh, 2005). Two in-vitro studies (Morelli *et al*., 1988; Igimi *et al*., 1996) have examined conjugative transfer in the gut microflora. A broad host range conjugative plasmid (pAMß1), was transferred into *L. reuteri* and *L. lactis*. These strains were then administered orally or by gastric tube to mice and the faecal contents were analysed to show plasmid transfer into *E. faecalis*. *Enterococci* spp. are known to be receptive for conjugation (Clewell and Weaver, 1989), but are also good at transferring antibiotic-resistant plasmids into Gram-negative (Courvalin, 1994) and Gram-positive bacteria (Perreten *et al*., 1997), at rates of $\log_{10}$ 10^{-4} to 10^{-9} transconjugants per recipient, in LAB (Mathur and Singh, 2005). While much attention has focused on antibiotic resistance in enterococci, more antibiotic

resistance transfer studies are required in non-enterococcal LAB, to examine their potential role in the dissemination of antibiotic resistant genes.

There are no reports linking conjugative transposons and antibiotic resistance in LAB, with the exception of those found in *E. faecalis*, (Tn916, Tn918, Tn920, Tn925, and Tn2702), *E. faecium* (Tn5233), *S. pyogenes* (Tn3701), and *S. agalactiae* (Tn93951). These transposons have been reported to carry resistance to tetracycline, erythromycin, chloramphenicol and kanamycin. Transposons also have the ability to mobilise unrelated plasmids or other chromosomal genes containing antibiotic resistance or virulence genes. The transfer (in-vitro) of tetracycline resistance (tet(M)) from *E. faecalis* to other LAB via a transposon has been reported to be between log10 10^{-6} to 10^{-9} transconjugants per recipient.

Impact of antimicrobial resistance in LAB

The widespread use of LAB and bifidobacteria in fermented food and dairy products has a safe history. While MDR is not common in these isolates, LAB like all other bacteria, are prone to gene exchange (Mathur and Singh. 2005). For these reasons, AMR will need to be monitored in these isolates, so that no starter or probiotic culture containing antibiotic-resistant determinants are used in food production.

Genetically Modified Organisms (GMOs)

Under EU law a genetically modified organism (GMO) is defined as an organism whose genetic material has been altered in such a way that does not occur naturally by mating and/or natural recombination (Commission for the European Communities, 2000). A number of concerns have been raised about the use of this technology including food safety issues, potential damage to the environment, disruption of ecosystems, as well as ethical and moral objections. In particular, questions have been raised about the relationship between antibiotic resistance and this technology. By linking an antibiotic resistant 'marker gene' to the 'gene of interest', only cells that incorporate the new genes into the bacterial cell will proliferate in the presence of an antibiotic, while cells which have not accepted the new genes fail to grow. Such a linkage has specific operational advantages in the detection, purification and cultivation of the "gene of interest", but may have negative effects in terms of the transfer and dissemination of AMR.

There are many ways in which GMOs can be produced, e.g., chromosomal alterations (Johansen, 1999), recombinant DNA methods (Curic *et al.*, 1999), or introduction of additional genes (Kondo and Johansen, 2002). Traditional cloning vectors normally contain antibiotic-resistance determinants as selectable markers, but these are considered unacceptable for food use. Initially food grade selection was based on nisin resistance (von Wright *et al.*, 1990), however intrinsic resistance to nisin in *L. lactis* prevented further development of this

The risk of the transfer of AMR genes from genetically modified organisms to gut micro-organisms is considered low.

marker. An alternative system is carried out by deleting a 'marker gene' present in the bacteria and reintroducing the same 'marker gene' on a plasmid (McCormick *et al*., 1995). However, this system has only been tested in laboratory strains. Another system which has been tested in industrial strains is the nonsense suppressor tRNA located on a plasmid which suppresses a nonsense mutation in the *pyrF* gene on the host chromosome. The disadvantage is that specific strains must be developed to act as a host for the food-grade vector. Strains over expressing aminopeptidases have been used successfully in small-scale cheese production (Dickley *et al*., 1995; Guldfeldt *et al*., 2001).

Impact of GMO's in antimicrobial resistance

The risk of the transfer of AMR genes to gut micro-organisms is considered low. However, the use of AMR markers is being phased out and replaced with other markers, such as those for herbicide resistance. Currently, only four types of GM crops may be found in food products on the Irish market including soya bean, rape seed, maize, and cotton seed), making up 0.5 per cent of the total ingredient/food (FSAI, 2004). All four crops are resistant to herbicides, with the latter two resistant to pest attack. This is in agreement with the 'Guidance document of the Scientific panel of Genetically Modified Organisms for the Risk Assessment of Genetically Modified Microorganisms and their Derived Products Intended for Food and Feed Use' (EFSA, 2006b), which recommends the use of alternative technologies that do not rely on antibiotic resistance markers. This guidance document also considers information about location and potential for transfer of each gene sequence. The phasing out of antibiotic-resistant marker genes will eliminate the potential for dissemination of resistance genes via GMO's in the future. On the request of the European Commission, the European Food Safety Authority (EFSA) issued an updated opinion in June 2009 on 'marker' genes, in particular antibiotic resistance marker genes, present in GMO's authorised in the EU. The panels concluded that, according to information currently available, adverse effects on human health and the environment resulting from the transfer of the two AMR marker genes, *nptil* and *aadA*, from GM plants to bacteria, associated with use of GM plants, are unlikely.

5

Food processing technologies

Evidence of increased resistance to food preservation stresses

Conjugation

Horizontal transfer of genes between different species of bacteria (Neuwirth *et al*., 2001, Winokur *et al*., 2001) is an evolutionary phenomenon whose extent and significance has been subject to intense debate. The maintenance and stability of transferred genes suggests that they confer a selective advantage on the recipient organism. In most cases the nature of this advantage is not understood, but the biological and clinical significance of the transfer of AMR genes remains clear (Koonin *et al*., 2002). Gene transfer experiments have been carried out successfully in several backgrounds including the intestines of various animals (Bourgeois-Nicolaos *et al*., 2006), in the human colon (Shoemaker *et al*., 2001) and in cultured human cells (Ferguson *et al*., 2002). Other environments investigated in a similar manner included bovine rumen fluid (Mizan *et al*., 2002), sewage (Ohlsen *et al*., 2003), surface water (Arvanitidou *et al*., 1998) and calf faeces (Yates *et al*., 2004). However, very little information is available on the transfer of AMRt genes in food matrices. While several studies have successfully demonstrated laboratory-based gene transfer by conjugation with food-borne strains in broth (liquid mating) (Allen and Poppe 2002; Chen *et al*., 2004; Wilks *et al*., 2005) or by filter (solid surface) mating (Pourshaban *et al*., 2002; Gevers *et al*., 2003), data describing the in-situ food matrix is limited (Cocconelli *et al*., 2003).

Walsh *et al*. (2008) reported the transfer of an ampicillin-resistance marker via an R-plasmid from S. Typhimurium DT104 to a susceptible recipient *E. coli* K12 in broth, milk and ground meat, at 25°C and 37˚C within 24-hour. A higher rate of transfer (10^{-2} cfu g^{-1} transconjugants per recipient) was reported in ground meat at 48 hour. Similarly, Van der Auwera *et al*. (2007) reported plasmid transfer (at 10^{-1} cfu ml/g^{-1} transconjugants per recipient) for *Bacillus thuringiensis* in broth, milk and milk pudding. Cocconelli *et al*. (2003), reported the transfer of vancomycin resistance via a conjugative R-plasmid in enterococcal strains during cheese and sausage fermentation. These authors

reported a 2-3 log (cfu g^{-1}) increase in the transfer rate of plasmids in meat, and suggested that factors including plasma in the meat matrix could be important. These differences may depend on the nature of the food matrix as human plasma, since plasma is reported to have a positive effect on plasmid transfer (Hirt *et al.*, 2002).

Another recent study by McMahon *et al.* (2007) showed increased rates ($P<0.05$) of resistance transfer under typical food processing stresses (high/low temperature, osmotic and pH stress), compared to controlled conditions (McMahon *et al.*, 2007). These effects could be related to increased activity or efficiency or one or more of the steps involved in resistance transfer, e.g. improved plasmid release/transfer or more efficient plasmid capture under stress. Selective pressures conferred by bacteriostatic, rather then bactericidal food preservation protocols, may be a driving force in the emergence of antibiotic resistance among zoonotic pathogens. However, to-date a knowledge gap exists on the relationship between food preservation stress and the transfer of antibiotic-resistance genes. For this reason, the mechanisms, incidence, and the epidemiological significance of stress-enhanced transfer of AMR remain unknown.

Transformation

Several bacterial species are naturally transformable, under a range of circumstances, including specific chemical or physical conditions (Davison *et al.*, 1999). Food processing can compromise the bacterial cell-membrane integrity enhancing the uptake of resistance genes on acquired extra-chromosomal DNA (Neu *et al.*, 1996). Transformation is enhanced by close contact with high concentrations of DNA and environments rich in nutrients, such as, biofilms (Baur *et al.*, 1996), frequent circumstances encountered in many parts of the food industry environment (Arnold and Silvers, 2000).

Increased natural competence of bacterial cells can be induced by salts, temperature shifts and electro-shocks (Davis *et al.*, 1999; Cérémonie *et al.* 2004; 2006), although DNA released from lysed bacterial cells is vulnerable to physical degradation during processing. For example, heat, shearing force, chemical degradation and natural DNA-ases present in food, such as, arginine, polyamines and biogenic amines (van den Eede *et al.* 2004; Weiss *et al.* 2007). Thus, food preservation technologies may injure cells enhancing DNA release, but also modify such DNA, allowing competent bacteria to acquire new or modified characteristics, such as antimicrobial resistance (IFT, 2006).

The development of competency and natural transformation has been demonstrated in *Bacillus subtilis* in milk (Zenz *et al.*, 1998; Kharazmi *et al.*, 2002). However the extent to which food processing contributes to the occurrence of transformation in food is unknown.

Transduction

While there are many reports of transduction of antibiotic-resistance genes within laboratory studies, there is no information available on transduction in the environment or in food. Its importance as a mechanism for gene transfer is questionable because of the high specificity of the phages required (Ammor *et al.*, 2007).

There is increasing concern that biocides may directly or indirectly stimulate the development and emergence of antimicrobial resistance by inducing sublethal stress.

6

Sanitizing Agents

Increased restriction and/or prohibition of the use of antibiotics as growth promoters, or hygiene aids, has necessitated an increase in the use of (alternative) biosecurity measures. These include the application of biocides to reduce microbial numbers which inevitably contaminate food processing equipment, surfaces and environments (Karatzas *et al*., 2007). These biocides differ from antibiotics in that they are usually broad-spectrum chemical agents, inhibiting or killing a wide range of micro-organisms by non-specific means (White *et al*., 2003). They are differentiated into disinfectants and sanitizers based on testing, claims and directions for use. Thus, a disinfectant must completely eliminate all the organisms listed on its label (including fungi and viruses), whereas a sanitizer need not eliminate all target organisms to be effective, nor are fungi and viruses ever included on a sanitizing claim.

Although biocides and antibiotics inactivate bacteria in very different ways, **there is increasing concern that biocides may directly or indirectly stimulate the development and emergence of antimicrobial resistance by inducing sublethal stress.** Thus their increasing application in food processing and domestic food preparation environments may be stimulating the development and dissemination of antimicrobial resistance in the human food chain. Biocide use included surface treatment with; alcohol, oxidizing compounds, hypochlorite, QACs, acid anionics, acidified sodium chlorite and chloride dioxide, and in hand care products e.g. triclosan, para-chloro-meta-xylenol and chlorhexidine (IFT, 2006).

Biocides

The efficacy of biocides and the types of organisms that they inhibit vary considerably and are dependent on the compositional concentrations and synergism among these components (Russell, 2000). In contrast to antibiotics, **biocides demonstrate a non-specific killing by coagulation of the cytosol and by damaging the cytoplasmic membrane. This is in marked contrast with the highly specific effects of antbiotics, many of which target very specific mechanisms within target cells**.

Biocide resistance

Resistance to biocides (disinfectants) is considered to be unlikely to occur, as most disinfectants are complexes of antimicrobial agents that inactivate multiple cellular targets (McDonnell and Russell, 1999; Russell, 2003). These compounds can also be applied at concentrations many times higher than the minimum inhibitory concentration (MIC). For this reason, decreased susceptibility does not always confer decreased bactericidal activity (Jones, 1999). Factors reducing the effectiveness of biocides include the presence of organic material and biofilm growth (Gilbert and McBain, 2001). **Inadequate disinfection procedures in livestock production facilities and food processing plants and household environments may contribute to the selection of biocide resistant isolates as a result of exposure to sublethal biocide concentrations**.

Reports of biocide resistance

Staphylococci showing decreased susceptibility to QACs have been isolated from food processing plants (Sundheim *et al*., 1998; Heir *et al*., 2004). Langsrud and Sundheim reported that more than 30 per cent of *Pseudomonas* spp. isolated from poultry carcasses can grow in the presence of benzalklonium chloride at concentrations used in the poultry plant (Langsrud and Sundheim, 2004). Resistance to QACs has been demonstrated in *Listeria* spp. isolated from poultry products, red meat and cheese (Lemaitre *et al*., 1998). In contrast, a recent report showed that biocide resistance was not a contributing factor to the persistence of strains of *L. monocytogenes* and *E. coli* in the food products and the environment of five chilled food production facilities (Hojah, 2002), suggesting that biocide resistance is only one of the factors involved in pathogen selection/survival. Little is known about the effects of low concentrations of biocides on bacterial biofilms. It may be of potential significance to the food industry that in-vitro studies found incomplete elimination of biofilm by biocides. This, in-turn, may lead to increased biocide resistance, due to the selection of highly resistant clones (McBain *et al*., 2003).

Biocides commonly used in the food industry and in domestic settings

Biocides commonly used in the food industry are listed in Table 12. Similar biocides are used in a domestic environment, in particular triclosan, QAC's, chlorhexidine (Braoudaki and Hilton, (2004a), hypochlorite (bleach) and chlorine (sometimes in combination with zinc) (IFT, 2006).

Inadequate disinfection procedures in livestock production facilities and food processing plants and household environments may contribute to the selection of biocide resistant isolates as a result of exposure to sublethal biocide concentrations.

Table 12 Biocides commonly used in the food Industry

Active ingredients	Environmental surfaces	Food contact surfaces	Food tissues	Restroom	Handcare
Alcohols	+	+			+
Oxidising compounds	+	+			
Hypochlorite	+	+	+	-	-
Quaternary ammonium compounds	+	+	+	+	-
Phenolics	-	-	-	+	-
Acid anionics	+	+	-	-	-
Acified sodium chlorite	+	+	+	-	-
Chlorine dioxide	+	+	+	-	-
Triclosan	-	-	-	-	+
Para-cholor-meta-xylenol	-	-	-	-	+
Chlorhexidine	-	-	-	-	+

Triclosan

Triclosan is commonly used in handcare products in the food industry. It works specifically on enoyl-acyl reductase, an enzyme which is essential for fatty acid synthesis. Modification, repression or deletion of the specific cellular target *fabI* (encoding enoyl-acyl reductase) results in reduced bacterial susceptibility to triclosan (McMurray, 1998a; Gilbert and McBain, 2003; Randall, 2004) in food pathogens (*E. coli*, *Salmonella* spp. and *Campylobacter* spp.) and food spoilage (*P. aeruginosa*) organisms. Additionally, many of the Resistance-Nodulation-cell Division (RND) family of pumps associated with resistance to clinically-important antibiotics are able to expel triclosan. These include the AcrAB-TolC pump of *E. coli* (McMurray, 1998b) and *Salmonella* spp. (Piddock, 2000), the CmeABC and CmeDEF of *C. jejuni* (Pumbwe *et al*., 2005), and several of the Mex pumps in *P. aeruginosa* (Chuanchuen *et al*., 2002).

Quaternary ammonium compounds

This group of biocide acts by physical disruption and partial solubilisation of the cell wall and membrane. For this reason, biocides are commonly used on environmental and food contact surfaces by the food industry. Resistance to QACs can be mediated through the action of nonspecific efflux pumps, along with other undesirable compounds from the interior of the bacterial cell. Coding for these pumps can be plasmid and chromosomally located, and both types have been described in both Gram-negative and Gram-positive bacteria (Gilbert and McBain, 2003). As stated earlier, the *qacEð1* is mapped to the conserved segment (3'-CS) of a class 1 integron structure. The chromosomal efflux determinants of QAC resistance in Gram-positive bacteria include the Major Facilitator (MF) family NorA multi-drug transporter (usually associated with fluoroquinolone resistance), the MF family MdeA, and the Multi-drug And Toxic Compound Extrusions (MATE) family MepA in *S. aureus* (Huang *et al*., 2004; Noguchi *et al*., 2004; Kaatz, 2005). The main mechanisms of QAC resistance is chromosomally mediated, whilst in Gram-positive bacteria it is plasmid-borne, due to the Small Multi-drug Resistance (SMR) family transporters QacC/D and QacEΔ1, QacG, QacH and QacJ and the MF family QacA/B transporter (Poole, 2007). In contrast, the transporters capable of exporting QACs in Gram-negative bacteria are generally chromosomally encoded and include a number of MATE (PmpM in *P. aeruginosa*), RND (AcrAB-TolC, AcrEF-TolC and YhiUV-TolC pumps of *E. coli*), (Poole, 2005) and SMR family (EmrE in *E. coli*) (Yerushlmi *et al*.1995) multi-drug transporters. The SMR transporters, QacE and QacEΔ1, QacF and QacG found in Gram-negative bacteria are plasmid-encoded (Paulsen *et al*., 1996; Poole, 2005).

Chlorhexidine

Chlorhexidine is used in hand-care products in the food industry. Bactericidal concentrations of chlorhexidine result in the denaturation of cytoplasmic proteins and coagulation of bacterial cell contents. The specific mechanism(s) associated with chlorhexidine resistance remain to be elucidated. However, chlorhexidine resistance is associated with *cepA*, which encodes a putative efflux mechanism in *K. pneumoniae* (Fang *et al*., 2002). Benzalkonium chloride and triclosan adapted *E. coli*, display a multi-drug-resistance phenotype including reduced susceptibility to chlorhexidine, consistent with increased expression of an RND multi-drug transporter (Braoudaki and Hilton,

The link between biocide exposure and the development of antibiotic resistance remains to be conclusively established.

2005). Chlorhexidine has been shown to induce expression of the MexCD-OprJ efflux pump in *P. aeruginosa* (Morita *et al.*, 2003) and QacA/B in Gram-positive bacteria reduces susceptibility to chlorhexidine (Morita *et al.*, 2003; Poole, 2005).

Evidence of links between antimicrobial and biocide resistance

The link between biocide exposure and the development of antibiotic resistance remains to be conclusively established. The theory of cross-resistance (to antibiotics or biocides) seems plausible; as resistance may be conferred by a shared mechanism (e.g. efflux pumps). Recent studies have shown that efflux pumps (sometimes with unusually broad specifcities) contribute to intrinsic resistance to agents, such as, antibiotics, dyes and detergents (Gilbert and McBain, 2003). Closer examination of the mechanisms involved and the potential role of efflux pumps might facilitate a clearer understanding of this relationship, and provide guidance on the appropriateness of such biocides, particularly in line with their expanding use.

Triclosan resistance

Many efflux pumps which confer resistance to triclosan also confer resistance to clinically-significant antibiotics. Karatzas *et al.* (2007) reported that in-*vitro* exposure to triclosan selected for *Salmonella* spp. with reduced antibiotic susceptibility arising from the overexpression of the AcrAB-TolC efflux pump. Similar studies have reported the same findings for *E. coli* (AcrAB-TolC) (Braoudaki, 2004a, Braoudaki, 2004b), *C. jejuni* (CmeABC and CmeDEF) (Randall *et al.*, 2007), *P. aeruginosa* (MexCD) (Chuanchuen *et al.*, 2001) and *S. maltophilia* (SmeDEF) (Sanchez *et al.*, 2005). While not all RND exporters may be significant determinants of triclosan resistance, their ability to export antibiotics and triclosan, highlights their potential contribution to the development of cross-resistance. The extent of this contribution requires epidemiological investigation (Poole, 2005).

No relationship between the use of triclosan and the development of antibiotic resistance has been reported in household studies (using products with/without triclosan) (Cole *et al.*, 2003). Moreover, a study conducted over a 10-year period found no relationship between triclosan use and antibiotic resistance in MRSA and *P. aeruginosa* (Marshall *et al.*, 2003). Since triclosan is effective against a wide range of bacteria, cross-resistance currently is not considered a problem (Poole, 2005).

Quaternary ammonium compounds and Chlorohexidine

Standard strains of *E. coli*, *Salmonella* spp. and *S. aureus* are highly sensitive to QACs and chlorohexidine and it is difficult to raise less susceptible subcultures (Russell, 2003). However, *in vitro* exposure to sub-lethal levels of QAC has been shown to select for MDR (arising from overexpression of the AcrAB-Tolc efflux pump) in *Salmonella* spp. and *E. coli* (Braoudaki *et al.* 2004a, 2004b; Karatzas *et al.* 2007). The relationship between chlorohexidine and RND transporters is not as well studied, as that of QAC's (Poole, 2005). Triclosan and QAC adapted *E. coli* isolates exhibit a MDR phenotype with reduced susceptibility to chlorohexidine (Braoudaki *et al.*, 2004a, 2004b). However, chlorohexidine adapted *E. coli* isolates

are not reported to exhibit an MDR phenotype (Poole, 2005). Unlike the strains previously discussed, *P. stutzeri* can be produced with markedly less susceptibility to chlorohexidine and QACs, in-*vitro*. These *P. seudomonas stutzeri* isolates demonstrate cross-resistance to some biocides and antibiotics (Tattawasart *et al*., 1999; Tattawasart *et al*., 2000a, 2000b). This cross-resistance is attributed to an alteration in the cell's outer membrane, resulting in a non-specific decrease in cell permeability (Russell, 2003).

Many epidemiological studies have found no relationship between biocide susceptibility and antibiotic resistance to biocides commonly used in hospital environments, including QACs and chlorohexidine (Alqurashi *et al*., 1996; Suller *et al*., 1999; Bacquero *et al*. 1991). However, a comparison of clinical and industrial isolates of *P. aeruginosa* revealed that clinical isolates had higher levels of resistance to antibiotics. This was attributed to the selective pressure of antibiotic use in hospitals. However, the adaptive response of *P. aeruginosa* to amikacin and tobramycin was accompanied by a low-level increase in tolerance to benzalkonium chloride (QAC) (Russell, 2003).

Numerous studies have clearly demonstrated that selection for biocide resistance can result in cross-resistance to antibiotics in-*vitro* (Moken *et al*., 1997, Braoudaki and Hilton, 2004a, 2004b). However, the true relationships between biocide use and antibiotic resistance, and the mechanisms involved, have as yet to be confirmed in epidemiological studies.

In addition to the health effects of AMR additional consequences are; infections that would not otherwise occur; increased frequency of treatment failures; and increased severity of infection.

7

Human health impact

Introduction

Humans can be exposed to antimicrobial-resistant bacteria through food derived from colonised animals, through increasing antimicrobial resistance in the environment and through food contamination during processing [see section 4]. Environmental contamination with resistant organisms, such as, surface waters, has also been reported, giving rise to concerns about direct exposure (Shea *et al*., 2004). An FAO/OIE/WHO meeting (Geneva in 2003) concluded that the emergence of antimicrobial-resistant organisms associated with non-human usage of antimicrobials posed adverse consequences to human health. More recently, **new EU regulations banning the use of antibiotics as growth promoters in animals have been introduced in an attempt to reduce the emergence of antibiotic resistance in the food-chain**.

Human health effects of antimicrobial resistance

A CDC review reported on the extra human health consequences of antimicrobial resistance as opposed to the health effects caused by susceptible organisms, and identified three additional consequences: infections that would not otherwise have occurred if the organisms were not resistant; increased frequency of treatment failures; and increased severity of infection (Angulo *et al*., 2004).

Infections that would not have otherwise occurred

When humans are taking antimicrobial medication, they are at increased risk of infection with resistant organisms, and in the US the number of additional infections as a result of resistant organisms in people taking antibiotics has been calculated (Barza and Travers, 2002). For instance, an additional 29,379 non-typhi salmonellae with 342 hospitalisations and 12 deaths were estimated to occur, and the excess cases for *C. jejuni* infections were calculated as 17,668 with 95 hospitalisations.

The increased severity of resistant infections has been demonstrated by greater hospitalisation rates.

Additional vulnerability to infection, for food animals receiving antimicrobials, may also result in an increase in infection and transmission from these animals (Angulo *et al.*, 2004).

In the US, more than 400,000 extra days of diarrhoea per year, and 8,677 extra days in hospital, have been reported as attributable to infection with resistant organisms, compared with infection with susceptible organisms (Travers and Barza, 2002).

Increased frequency of treatment failures

In a S. Typhimurium DT104 outbreak, two deaths in patients treated with fluoroquinolones were considered flouorquinolone-resistance-related by the coroner (Angulo *et al.*, 2004). In the same review, it was reported that patients taking fluoroquinolones, who have resistant *Campylobacter* infection, have been found to have several additional days of diarrhoea.

A potential lack of therapeutic options is a real concern, for example, the drug of choice in the treatment of children with invasive *Salmonella* spp. disease, ceftriaxone, was not an option in a child with ceftriaxone-resistant strain of *Salmonella* spp. associated with the same organism in a local outbreak of salmonellosis in cattle (Fey *et al.*, 2000).

Increased severity of infection

The increased severity of resistant infections has been demonstrated by greater hospitalisation rates, and greater case fatality rates (Angulo *et al.*, 2004). Increased risk of bloodstream infections has also been seen, as well as death within 90 days (Angulo *et al.*, 2004).

Mortality associated with infections with resistant organisms

Helms *et al.* (2002) found an increased risk of death over two years in those infected with S. Typhimurium –59 out of a total of 2047 patients died as a result of S. Typhimurium. Even for susceptible *Salmonella* spp. the risk of death was 2.3 times more than for the general Danish population (adjusted for age and co-morbidity). With multi-resistance including quinolone resistance, the risk of death increased by a factor of 10.3 (95 per cent CI 2.8-37.8) (Helms *et al.*, 2002).

Changes increasing the vulnerability of host populations

As survival times following cancer treatment, transplantation and other chronic diseases, such as diabetes mellitus and autoimmune diseases increases so does the pool of immuno-suppressed patients alive in the community. Improved life expectancy and the greater survival of premature infants also increase the population of those most vulnerable to any infections, and particularly resistant infections.

Economic and other costs to health services

In the US, varying proportions of antimicrobial use for veterinary purposes have been reported: from 40 per cent of all antimicrobials used, with only about a quarter of this used for therapeutic reasons to 78 per cent of all antimicrobials use being used in food animals for non-therapeutic reasons (Shea *et al.*, 2004). This indicates that antimicrobial agent use in food animals is a sizable problem and its side effects may present a major challenge to veterinary public health.

Human healthcare is costly and as a result, resources must be managed in the most cost-effective manner. Efforts to control antimicrobial resistance through prudent use of antibiotics and through 'search and destroy' policies are threatened by imprudent use in human and animal healthcare. In The Netherlands, for instance, MRSA prevalence in farm families is much higher than in the general population, where human healthcare policies have been successful and this new source of antimicrobial resistance is outside the direct influence and control of human health services.

Options are reduced in the treatment of AMR resistant infections requiring the use of more expensive and often toxic drugs. So even if an effective alternative treatment is available, this potentially avoidable cost could be prevented.

Infected individuals are a source of infection for others, so prolonged illness, as occurs with many resistant organisms, increases the cost of isolation and other control measures. There is also an increased risk of cross-infection and outbreaks, which apart from the significant human costs would escalate the economic impacts of healthcare. This is very important as hospitals are populated by particularly vulnerable people. Other complicating factors need to be considered, for example, increased hospital stays lead to increased bed occupancy and possible overcrowding, which increases the risk of cross-infection.

Increased morbidity and mortality, again apart from the human cost to patients and their families, are potential sources of litigation, which even if negligence is not proven, are costly to defend.

Opportunity costs – increased costs for healthcare, longer hospital stays, more intensive therapy, more expensive treatments, more complications, more after-care divert the use of scarce resources from other important health needs, thereby using resources for other healthcare provision, such as, cancer treatments which cannot always be afforded.

Human antimicrobial resistance surveillance:

There are two basic elements to human AMR data, antimicrobial consumption data and data on pathogen testing. There are well-recognised limitations to the data sets both in terms of accuracy and completeness. Some of these include: lack of antimicrobial prescription, sale and consumption data; only a limited number of clinically important pathogens are tested for resistance; sampling strategies and laboratory methods are not standardised and not all isolates are tested for all antimicrobials. A detailed review of the available data is outside the scope of this document. However, relevant data can be sourced from the following websites: ESAC (EU Surveillance of Antibiotic Consumption), ECDC, EARSS, CDSCNI and HPSC.

Both SARI and AMRAP have recommended the development of guidance in relation to the appropriate use of antimicrobials (in humans), as well as monitoring the supply and use of antimicrobials in hospitals and the community.

8

Solutions

Prudent use of antimicrobial agents

On the island of Ireland there are two AMR strategies, namely SARI (Strategy for the control of Antimicrobial Resistance) in ROI and AMRAP (Antimicrobial resistance Action plan) in NI. Both SARI and AMRAP have recommended the development of guidance in relation to the appropriate use of antimicrobials (in humans), as well as monitoring the supply and use of antimicrobials in hospitals and the community (NDSC, 2001). Education in relation to the human health use of antimicrobials and hygiene were also recommended and some materials have been developed to assist with this. There have also been two joint conferences aimed at relevant professionals.

However, success in the implementation of prudent prescribing of antimicrobials in human healthcare is complex. Research has been undertaken on prescribing practices focusing on financial cost (Parrino, 1989), knowledge and attitudes of physicians (Poses and Anthony, 1991; Ehedahl *et al.*, 1995) and the attitudes of patients or parents towards the inappropriate use of antimicrobials (Palmer and Bauchner, 1997).

The most recent evidence indicates that even when the need for rational prescribing is accepted, there are ongoing challenges to achieve best practice.

In veterinary practice, international guidelines on the responsible and prudent use of antimicrobials have been developed by the Office International des Epizooties (Anthony *et al.*, 2001).

According to a review by Collignon (Collignon 2004), there is an absence of evidence of any significant benefit to agriculture from the use of antimicrobials as growth promoters. Prohibiting the use of antimicrobials as growth promoters has a major effect on reducing the pool of resistant organisms with minimal effect on productivity (Wegener, 2003). Alternative animal health care strategies have been proposed, for example, because of resistance clustering within herds, prevention strategies at herd level interventions (Rosengren *et al.*, 2008).

The European Food Safety Authority (EFSA) has reported that:

"in terms of impact, controls operated at the pre-harvest phase, for example, those aimed at the control and limitation of antimicrobial usage are potentially the most effective and as such are capable of playing a major role in reducing the occurrence of antimicrobial resistant bacteria in food as presented for sale" (Scientific Opinion of the Panel on Biological Hazards on a request from the EFSA on foodborne antimicrobial resistance as a biological hazard, 2008).

Need for on-going surveillance

Even with strict adherence to guidelines, as long as antimicrobials are used at all, resistance will remain a threat and, therefore, there is a need for on-going human and animal surveillance, both to monitor the outcomes of co-ordinated control efforts and for the early identification of new resistance threats.

EFSA has recommended:
"the development and application of new approaches to the recognition and control of food as a vehicle for antimicrobial resistant bacteria and related genes based on epidemiological and source attribution studies directed towards fresh crop-based foods, raw poultry meat, raw pigmeat and raw beef" (Scientific Opinion of the Panel on Biological Hazards on a request from the EFSA on foodborne antimicrobial resistance as a biological hazard, 2008).

The existing human AMR surveillance systems are briefly described in section 8 of this report.

In animal health surveillance there is a less well developed standardised surveillance system across Europe. Surveys have been carried out in local areas. In the ROI for example, monitoring of in-line milk filters found *Salmonella* infection in six per cent of herds, and all strains found were resistant to at least one antibiotic (Murphy *et al.*, 2008).

The Community Summary Report on Trends and Sources of Zoonoses Zoonotic Agents and Antimicrobial Resistance in the European Union was first published by EFSA in 2006.

Surveys have also been carried out internationally, showing carriage of antimicrobial resistance across species and across countries (Bywater *et al.*, 2004).

A model for continuous surveillance for antimicrobial resistance among isolates from food animals exists since 1995 in Denmark (Aarestrup, 2004). The benefits of the Danish surveillance system are felt internationally as this source of surveillance data has informed international scientific knowledge in recent years. However, Aarestrup recommends: "an organised monitoring of antimicrobial resistance carried out by an international network" (Aarestrup, 2004).

Structured surveillance systems which integrate animal, human and food chain surveillance across jurisdictions, are necessary to ensure effective public health planning and intervention across the island of Ireland.

9

Recommendations

The recommendations below are grouped in to two categories:- surveillance and research issues. The surveillance issues focus on the crucial inter-locking role of ***safe*food** between veterinary and clinical medicine, to achieve an overall quality of data for the people on the island of Ireland. The research issues reflect specific research areas in support of the particular role of ***safe*food** in consumer protection.

Surveillance issues:

- Current levels and intensities of AMR surveillance vary widely between the animal population, the food chain, and the human population. Much of such work is episodic, with little or no ongoing surveillance or wider harmonization across the island. Structured surveillance systems which integrate the above aspects, across jurisdictions, are necessary to ensure effective public health planning and intervention across the island of Ireland.
- An integrated AMR monitoring programme, with a clear emphasis on those areas that are currently underdeveloped, i.e. food-borne AMR, should be developed on the island of Ireland (e.g. DANMAP model). Food safety in relation to AMR should be informed by risk assessment of the impact of environmental exposure to antimicrobial agents.
- Integrated monitoring of antimicrobial prescribing, dispensing and consumption patterns in human and animal populations on the island of Ireland is required. These monitoring systems should be linked and ongoing.
- Programmes promoting the prudent use of antimicrobial agents in animal and human medicine on the island of Ireland should link with all Food Safety agencies. Current programmes promoting the prudent use of antimicrobial agents (in both jurisdictions) should be supplemented and extended, to ensure adequate coverage in relation to the food production, processing, and retail chain.
- A Forum on Food Safety and AMR should be established. Relevant stakeholders should include policy makers, food industry, food scientists, environmental protection agencies and medical and veterinary professionals (a

'One Medicine' approach). The forum should be a collaborative network and have the capacity to hold conferences, issue reports, and act to ensure improved public safety in relation to the risks posed by AMR bacteria in the human food chain.

Research issues:

- Research into the potential dangers and impacts of AMR on current and alternative food technologies should be conducted in association with food industry partners. Initially, this work should focus on technologies such as minimal processing and probiotic supplementation of food products.
- Food attribution studies should be carried out to estimate the fraction of food animal associated AMR infections on the island of Ireland with a view to supporting ongoing identification and prioritisation of hazards posed by (and interventions for the reduction of) such AMR. These should be stratified by populations and regions on the island of Ireland, as the attributable fractions in different communities, e.g.: urban versus rural, are unlikely to be the same.

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Appendix

Table 1 Classes of antimicrobials, examples of substances used in human and veterinary medicine and examples of resistance genes. (Modified from EFSA, 2007 – Opinion on foodborne antimicrobial resistance as a biological hazard).

Note: There is generally complete or partial cross resistance within each class or subclass unless otherwise indicated.

See Table 1 over leaf.

Class	Examples of substances used in:			
	Human medicine	**Veterinary medicine; food production animals in EU**	**Resistance genes**	**Comments**
Aminoglycosides	amikacin, gentamicin, netilmicin, tobramycin	apramycin, gentamicin, streptomycin	*aac, aad (ant), aph, armA, rpsL (strA) rpsD, rpsE, strB*	No general cross-resistance within class, but some types of resistance will involve cross-resistance between some aminoglycosides
	kanamycin, spectinomycin	neomycin, spectinomycin		
Amphenicols	chloramphenicol, tiamphenicol	chloramphenicol, florfenicol, tiamphenicol	*cat, cfr, cml, flo*	crf confers cross-resistance to amphenicols, lincosamindes, pleuromutilins, streptogramins, linezolid
Beta-lactam antibiotics				
Penicillins	Benzyl-penicillin, ampicillin, amoxicillin (with clavulanic acid)	benzyl-penicillin, ampicillin, amoxicillin (with clavulanic acid)	*blaZ (bla-PC), bla-TEM. bla-SHV*	Cross resistance within sub-classes but also, depending on mechanism, between subclasses

Penicillins – antistaphylococcal	cloxacillin, dixcloxacillin (methicillin)	cloxacillin, dicloxacillin	*bla-OXA, mecA*	
Cephalosporins, first generation	cephalexin, cefazolin, cephalotin	cefazolin, cephalexin	*bla-TEM. bla-SHV, bla-CTX, bla-CMY, some bla-OXA*	
Cephalosporins, second generation	cefuroxime, loracarbef	-		
Cephalosporins, third generation	ceftazidime, ceftriaxone	Ceftiofur		
Cephalosporins, fourth generation	cefepime, cefpirome	cefepime, cefquinome		
Cephamycins	cefoxitin		*bla-CMY, bla-AAC*	
Carbapenems	ertapenem, imipenem, meropenem	-	*bla-IMP, bla-VIM, some bla-OXA*	
Cyclic polypeptides	Bacitracin	(bacitracin)	*bcrABD*	Formerly used as feed additive in EU
Glycopeptides	teicoplanin, vancomycin	- (avoparcin)	*van (A-E)*	Formerly avoparcin was used as feed additive in EU

Ionophores	-	monensin, salinomycin		Used as coccidiostats
Lincosamides	clindamycin, lincomycin	clindamycin, lincomycin	*cfr, erm*	Cross-resistance also to macrolides and streptograminB for certain resistance genotypes
Lipopeptides	daptomycin	-		
Macrolides & ketolides	erythromycin, spiramycin, azithromycin, clarithromycin	spiramycin, tylosin, tulathromycin	*erm, ere, mef, msr*	Cross-resistance also to lincosamides and streptograminB for certain resistance genotypes
Nitrofurantoins	furazolidone, nitrofurantoin	-		Used formerly as veterinary medicine
Nitroimidazoles	metronidazole, tinidazole	-		Dimetridazole and ronidazole used formerly as veterinary medicine
Orthosomycins	-	Avilamycin	*emtA*	Used formerly as feed additive
Oxazolidones	linezolid	-	*cfr*	

Pleuromutilins	-	tiamulin, valnemulin	*cfr*	
Polymixins	colistin, polymixin B	colistin, polymixin B		
Quinolones	nalidixic acid, ciprofloxacin norfloxacin, moxifloxcin	danofloxacin, enrofloxacin,	*aac(6')-Ib-cr, gyrA. parC, qepA, qnr,*	Incomplete cross-resistance
Quinoxalines	-	carbadox, olaquindox	*oqxAB*	Olaquindox used formerly as feed additive in the EU
Streptogramins	pristinamycin, quinpristin/ dalfopristin	- (virginiamycin)	*cfr, erm, vga , vgb*	Formerly virginiamycin was used as feed additive in EU Cross-resistance between streptograminB, lincosamides and macrolides for certain resistance genotypes
Sulphonamides & trimethoprim	sulfadiazine, sulfamethoxazole, trimethoprim	sulfadiazine, sulfadoxine, sulfamethoxazole, trimethoprim	*dfr, sul*	

Tetracyclines	chlortetracycline, doxycycline, oxytetracycline	chlortetracycline, doxycycline, oxytetracycline	*tet*	
Miscellaneous	-	flavophospholipol (bambermycin)		Used formerly as feed additive
	fosfomycin	-	*fosAB*	
	fusidic acid	fusidic acid	*fusB*	
	Mupirocin	-	*mupA*	Used in human medicine topically for MRSA decontamination
	Rifampicin	(rifampicin)	*rpoB*	Use in vet. med limited to foals
Oxazolidones	linezolid	-	*cfr*	

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