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Antibiotic-Resistant Bacteria: A Challenge for the Food Industry

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Antibiotic-resistant bacteria were first described in the 1940s, but whereas new antibiotics were being discovered at a steady rate, the consequences of this phenomenon were slow to be appreciated. At present, the paucity of new antimicrobials coming into the market has led to the problem of antibiotic resistance fast escalating into a global health crisis. Although the selective pressure exerted by the use of antibiotics (particularly overuse or misuse) has been deemed the major factor in the emergence of bacterial resistance to these antimicrobials, concerns about the role of the food industry have been growing in recent years and have been raised at both national and international levels. The selective pressure exerted by the use of antibiotics (primary production) and biocides (e.g., disinfectants, food and feed preservatives, or decontaminants) is the main driving force behind the selection and spread of antimicrobial resistance throughout the food chain. Genetically modified (GM) crops with antibiotic resistance marker genes, microorganisms added intentionally to the food chain (probiotic or technological) with potentially transferable antimicrobial resistance genes, and food processing technologies used at sub-lethal doses (e.g., alternative non-thermal treatments) are also issues for concern. This paper presents the main trends in antibiotic resistance and antibiotic development in recent decades, as well as their economic and health consequences, current knowledge concerning the generation, dissemination, and mechanisms of antibacterial resistance, progress to date on the possible routes for emergence of resistance throughout the food chain and the role of foods as a vehicle for antibiotic-resistant bacteria. The main approaches to prevention and control of the development, selection, and spread of antibacterial resistance in the food industry are also addressed.

Keywords Antibiotic-resistant bacteria, food safety, food industry, antimicrobials, antibiotics, biocides, genetically-modified plants, probiotics, technological treatments

ABBREVIATIONS

AHI = Animal Health Institute (United States)
 AMR = antimicrobial resistant
 ASC = acidified sodium chlorite
 BIOHAZ = EFSA Panel on Biological Hazards
 BOD = biological oxygen demand
 CDC = Centers for Disease Prevention and Control (United States)
 CE = competitive exclusion
 CHX = chlorhexidine diacetate
 COD = chemical oxygen demand
 DNA = deoxyribonucleic acid
 DT = definitive phage type
 EARSS = European Antimicrobial Resistance Surveillance System

EASAC = European Academies Science Advisory Council
 EC = European Communities
 ECDC = European Centre for Disease Prevention and Control
 EEA = European Environment Agency
 EFSA = European Food Safety Authority
 EHEC = enterohaemorrhagic *Escherichia coli*
 EMEA = European Medicines Agency
 ESBLs = extended spectrum beta-lactamases
 EU = European Union
 FAO = Food and Agriculture Organization
 FDA = Food and Drug Administration (United States)
 FEEDAP = EFSA Panel on Additives and Products or Substances used in Animal Feed
 FVE = Federation of Veterinarians of Europe
 GHP = good hygiene practices
 GM = genetically modified
 GMM = genetically modified microorganism
 GMO = genetically modified organism
 GMP = good manufacturing practices

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GPA	= growth promoter antibiotics
GRAS	= generally recognized as safe
HACCP	= hazard analysis and critical control point
HIV	= human immunodeficiency virus
HPA	= Health Protection Agency (United Kingdom)
ICU	= intensive care unit
IFT	= Institute of Food Technologists (United States)
MDR	= multidrug-resistant
MIC	= minimum inhibitory concentration
MRL	= maximum residue limits
MRSA	= methicillin-resistant <i>Staphylococcus aureus</i>
NARMS	= National Antimicrobial Resistance Monitoring System (United States)
NNIS	= National Nosocomial Infection Surveillance (United States)
OIE	= Office International des Epizooties (World Organisation for Animal Health)
OJEC	= Official Journal of European Communities
OJEU	= Official Journal of European Union
PABA	= <i>para</i> -aminobenzoate
PBPs	= penicillin-binding proteins
PEF	= pulsed electric field
QAC	= quaternary ammonium compound
QPS	= qualified presumption of safety
RASFF	= Rapid Alert System for Food and Feed (European Union)
RNA	= ribonucleic acid
SCAN	= Scientific Committee for Animal Nutrition (Czech Republic)
SCENIHR	= Scientific Committee on Emerging and Newly Identified Health Risks (European Union)
SCHER	= Scientific Committee on Health and Environmental Risks (European Union)
UK	= United Kingdom
USA	= United States of America
VRE	= vancomycin-resistant enterococci
WHO	= World Health Organization

INTRODUCTION

Since their discovery, antibiotics and related medical drugs have led to an important drop in deaths from infectious diseases, and have contributed to the major gains in life expectancy experienced during the latter part of the last century (Armstrong et al., 1999). However, in recent decades, these gains have been jeopardized by the emergence and spread of antibiotic-resistant microbes, which have challenged clinicians and researchers. At present, antibiotic resistance is a global public health threat that involves all major microbial pathogens and antimicrobial drugs, affecting both current and future generations.

Concerns about bacterial drug resistance from bacterial pathogens have been growing for a number of years and have been raised at both national and international levels (EARSS, 2005; EASAC, 2007; EFSA, 2007a; Jansen et al., 2006; WHO,

2007a). Thus, antimicrobial resistance has been defined as a global pandemic (EASAC, 2007), one of the major global public health threats, one of the major sanitary challenges of the twenty-first century (WHO, 2009), a potential worldwide catastrophe (European Parliament, 2006), and a major issue in European health care (ECDC/EMEA, 2009). According to recent trends in mortality, predictions using logistic models suggest that during the twenty-first century, infections could gradually regain their fatal edge of a century ago (Ausubel et al., 2001). The problem is so severe that many experts believe that the value of antibiotic therapies over the next 100 years is uncertain (Rosenblatt-Farrell, 2009).

History and Trends in Antibiotic Resistance

Sir Alexander Fleming, returning from his summer holidays in September 1928, discovered penicillin by looking at an agar plate where the growth of a contaminant mold had inhibited growth of *Staphylococcus aureus*. The mold was later identified as *Penicillium notatum* and the chemical responsible for inhibition was named penicillin. Purification, production in sufficient quantities, and use of penicillin in patient treatment were performed in 1940 by a group of researchers at Oxford University (particularly Ernst Boris Chain and Howard Walter Florey). These three scientists received the Nobel Prize for Physiology and Medicine in 1945 (Monnet, 2005).

Antibiotic resistance was described soon after, and in an interview with the New York Times in 1945 Fleming warned that the inappropriate use of penicillin could lead to the selection of resistant "mutant forms" of *Staphylococcus aureus* that could cause more severe infections in the host or in other people with whom the host has been in contact, and could thus pass on the resistance microbe. He was right, and within one year of the widespread use of this drug a significant number of strains of this bacterium had become resistant to penicillin. Only a few years later, over 50% were no longer susceptible to this new drug (Alanis, 2005).

Data on antibiotic-resistant bacteria in invasive health-care associated infections (mainly bloodstream infections) are available from the European Antimicrobial Resistance Surveillance System (EARSS) for EU Member States, Iceland and Norway for each year during the period 2002–2007. The proportion of various antibiotic-resistant species among blood isolates, especially Gram-negative bacteria (e.g., *Escherichia coli* resistant to cephalosporins), has been rising steadily over this period. In the European Union, resistance to antibiotics is high among bacteria that cause severe infections in humans and reaches 25% or more in several EU Member States (EARSS, 2007; ECDC/EMEA, 2009). Of great concern are multidrug-resistant strains, which are responsible for half of the approximately 27,000 deaths a year from healthcare-associated infections in the 27 member states of the European Union (Watson, 2008). Various other surveillance systems available in the European Union also show a general trend towards an increase in the prevalence of

antibiotic resistance for most enteric food-borne pathogenic bacteria (ECDC/EFSA/EMEA/SCENIHR, 2009; European Commission, 2006a; HPA, 2008).

In the USA, the prevalence of antimicrobial resistance has increased markedly in recent years, both for enteric (NARMS, 2006) and health-care associated infections (CDC, 2009). For example, methicillin-resistant *Staphylococcus aureus* (MRSA), a healthcare-associated microorganism that may also be transmitted by eating and handling contaminated foods (EFSA, 2009a), was first isolated in the United States in 1968. By the early 1990s, MRSA accounted for 20% to 25% of *Staphylococcus aureus* isolates from hospitalized patients. In 1999, MRSA accounted for more than 50% of *S. aureus* isolates from patients in Intensive Care Units (ICUs) in the National Nosocomial Infection Surveillance (NNIS) system; in 2003, 59.5% of *S. aureus* isolates in NNIS ICUs were MRSA. A similar increase in prevalence has occurred in vancomycin-resistant enterococci (VRE). VRE causes nosocomial infections, and can also be transmitted from animals through ingestion or direct contact, colonizing human beings and sometimes causing infection (ECDC/EFSA/EMEA/SCENIHR, 2009; Peters et al., 2003). From 1990 to 1997, the prevalence of VRE in enterococcal isolates from hospitalized patients increased from less than 1% to approximately 15%. VRE accounted for almost 25% of *Enterococcus* isolates in NNIS ICUs in 1999, and 28.5% in 2003. Gram-negative bacteria resistant to extended spectrum beta-lactamases (ESBLs), fluoroquinolones, carbapenems, and aminoglycosides have also increased in prevalence. For example, in 1997, the SENTRY Antimicrobial Surveillance Program found that among *Klebsiella pneumoniae* strains isolated in the United States, resistance rates to ceftazidime and other third-generation cephalosporins were 6.6%, 9.7%, 5.4%, and 3.6% for bloodstream, pneumonia, wound, and urinary tract infections, respectively. In 2003, 20.6% of all *K. pneumoniae* isolates from NNIS ICUs were resistant to these drugs. In the period 1994 to 2000, a national review of Gram-negative bacteria isolated from

ICU patients in 43 states found that the overall susceptibility to ciprofloxacin decreased from 86% to 76% and was temporally associated with increased use of fluoroquinolones in the United States (CDC, 2006).

Most threatening of all are diseases caused by bacteria resistant to virtually all currently available drugs (pan-resistant strains). The increasing development of such organisms raises the specter of a post-antibiotic era, and has the potential to become a worldwide catastrophe (European Parliament, 2006).

Trends in Antibiotic Development

It is clear that the need for new antibiotics is greater than ever before, because of the emergence of multi-drug-resistant and pan-resistant pathogenic microorganisms, the rapid emergence of new infections, and the potential for use of multidrug-resistant agents included in bio-weapons (Spellberg, 2008). Paradoxically, despite the clinical need for new antimicrobial agents, the development of these agents is declining. Since the 1970s, few new antibiotics have been discovered, and experts estimate that the chance of finding new antibiotics is small. This is partially because little research into new antibiotics is being conducted and partly, and perhaps more worryingly, because there may be few remaining effective antibiotics to be discovered (Becker et al., 2006; ECDC/EMEA, 2009).

Costs for pharmaceutical research and development, which are estimated to be about \$800 million to \$900 million and 10 to 15 years per approved agent (Monnet, 2005), combined with the very stringent approval criteria adopted in recent years by several regulatory bodies, pose a considerable barrier to the development of new drugs in general. Moreover, antimicrobial agents are less economically attractive targets for development than other drug classes (Table 1) (Carpenter and Chambers, 2004; Collier and Iheanacho, 2002; Monnet, 2005; Spellberg et al., 2004; Trémolières et al., 2010). More than ever before,

Table 1 Main causes for declining development of antibiotic drugs

1. Developing an antibiotic is potentially more difficult than for other drugs because the mode of action might differ from one bacterial species to another, and the new drug must be tested against all species.
2. The fact that the population of developed countries is ageing means that it is more profitable to develop treatments for medical conditions which are more prevalent among elderly people and require life-long daily treatment, such as hypercholesterolemia, hypertension, depression, dementia, and arthritis than to develop antibiotics. For example, in 2000, amoxicillin-clavulanate was the only antibiotic in the list of the top 20 prescription drugs in the USA, and its sales were about one-third of cholesterol-lowering drugs (Kreling et al., 2001).
3. Research and development efforts are gradually being increasingly channelled into conditions that are very profitable for pharmaceutical companies in developed countries, such as baldness or inadequate sexual performance, which require specific treatments (Courvalin and Davies, 2003).
4. Antimicrobials are usually used for short-course therapies (5–14 days) that cure disease, thus eliminating their need for long-term use in a given patient.
5. Increasing concern about overuse and misuse among physicians and the general public has led to a general decrease in antibiotic use in developed countries.
6. The appropriate public health need to limit use of broad-spectrum antimicrobials and thereby minimize the pressures driving resistance, has caused the medical community to discourage the first-line use of newly developed antimicrobials, negatively impacting sales.
7. There is increasing pressure from health care insurance systems to use fewer and cheaper antibiotics, and despite renewed alerts about emerging resistance, most infections are still treatable with existing antibacterial drugs. New agents specifically launched to target resistance (e.g., linezolid and quinupristin-dalfopristin) have not captured the market for which they were projected (Projan, 2003).
8. The large number of antimicrobials already approved implies a high level of competition for newly developed agents. The new antibiotic must be as effective as existing ones against susceptible strains, but must also be effective against bacterial strains that have acquired resistance for existing drugs.
9. Antibacterial drugs have a short life-span, because resistance to a new agent will eventually develop in connection with the commercialization and use of any new antibacterial drug.

prospective investments in antibiotics are competing with drugs for muscle-skeletal and neurological disease with 10 or 15 times more “net present value,” a measure used in the industry to predict the potential success of products. Thus, among existing antibacterial drugs, only a few have had sufficient turnover to recoup research and development costs (Bax, 2001). Prioritizing measures to secure optimal returns and investment have driven the industry into other pharmaceutical areas with larger and safer markets.

Spellberg et al. (2004) have reported that FDA approval of new antibacterial agents (new molecular entities) decreased by 56% over the past 20 years: 1983 to 1987 (16 agents) vs 1998 to 2002 (7 agents). Moreover, projecting future developments, new antibacterial agents constitute about 1% (6 out of 506) of drugs disclosed in the development programs of the five largest pharmaceutical and biotechnology companies. These companies direct less than 2% of their research resources to developing new antibacterial drugs. Moreover, pharmaceutical companies are still focusing on extending the antibacterial spectrum of existing compounds by means of semi-synthetic optimization, or combining existing antibacterial compounds, rather than trying to find new chemical structures that could lead to new classes of antibacterial agents (Monnet, 2005).

Several mechanisms to encourage industrial drug research and development have been reported (ECDC/EMA, 2009; Webber and Kremer, 2001). However, even if the pharmaceutical industry and public and university laboratories were to step up efforts to develop new drugs immediately, current trends suggest that some diseases will have no effective therapies within the next ten years.

Consequences of Antibiotic Resistance

Infections caused by resistant bacteria fail to respond to treatment, resulting in increased economic cost, as well as severe consequences associated with morbidity and mortality (Rice, 2009).

Economic Aspects

Infections by resistant pathogens cause a financial burden to hospitals, healthcare systems, and societies through the exacerbation or prolongation of illness and subsequent in-hospital treatment, with potentially crucial health consequences for the infected individual (Table 2). Estimates suggest that high costs to the medical care sector for resistance go back as far as 1995, with a cost in excess of \$4,000 million to \$5,000 million in that year for the United States. Annual costs to the medical care sector in Europe have been estimated more recently and suggest that unrecognized costs associated with antibiotic consumption in 2001 were €9,000 million, excluding the costs associated with antibiotic prescriptions in hospitals (European Parliament, 2006). More modest estimates (\$1,300 million to \$2,700 million in the USA and \$1,500 million in the EU) have recently been reported, although it is thought that most studies represent underestimates (ECDC/EMA, 2009).

Health Aspects

Antimicrobial-resistant bacteria can have human health consequences both because of the occurrence of infections that would otherwise not have occurred, and because of treatment

Table 2 Economic consequences associated with antibiotic resistance

1. Additional investigations such as laboratory tests and X-ray examinations.
2. Higher (at least two-fold) probabilities of hospitalization and longer hospital stay than for sensitive strains.
3. Additional or alternative (second or third-line drugs) treatments, often much more expensive than drugs used to treat infections caused by sensitive organisms (first-line treatments).
 - For example, changing treatment from oral oxacillin/dicloxacillin for susceptible infections to intravenous vancomycin treatment for resistant *Staphylococcus aureus* infections increases the cost by a factor of 5–10 (European Parliament, 2006).
 - The drugs needed to treat multidrug-resistant forms of tuberculosis are over 10–100 times more expensive than the first-line drugs used to treat non-resistant forms. In many countries, the high cost of such replacement drugs is prohibitive, which implies that some diseases can no longer be treated in areas where resistance to first-line drugs is widespread (WHO, 2002). It has been estimated that a resistant *Mycobacterium tuberculosis* strain doubles the cost of standard treatment (\$13,000 vs \$30,000), and in tuberculosis caused by a multidrug-resistant strain, treatment costs increase to \$180,000 (Rajbhandary et al., 2004; Wilton et al., 2001).
 - Substantially higher costs are involved for pneumonia treatment caused by a non-penicillin-susceptible *Streptococcus pneumoniae* than for a susceptible strain. Higher costs are due to longer hospital stay (26.8 vs 11.5 days) and more expensive medicines (\$736 vs \$213) (Einarsson et al., 1998).
 - The medical costs attributable to antimicrobial resistant infections in a hospitalized patient range from \$18,588 to \$29,069 (EASAC, 2007; Roberts et al., 2009).
4. Additional side-effects from more toxic treatments, which have to be managed.
5. Longer time off work.
6. Increased burden on family or infected individual.
7. Additional costs for hospital when hospital-acquired infections occur and infection control procedures are required. Cost to a general hospital of containing a 5-week outbreak of methicillin-resistant *Staphylococcus aureus* (e.g., hygienic measures) has been estimated to be about 600,000 € (Cox et al., 1995).
8. Increased overall healthcare expenditure, including costs of combating transmission.
9. Increased costs of disease surveillance.
10. Increased costs to firms and absenteeism.
11. Possible increase in product prices due to increased cost to firms.

failures and increased severity of infections. It is difficult to quantify precisely the total impact of antibiotic resistance in terms of morbidity and mortality, because resistance constitutes a problem in addition to the initial infection. However, it is clear that patients are more likely to die if they are infected with an antibiotic-resistant bacterium and will, if they do survive, have required more expensive therapy, have been sick for a longer time period and have been more likely to require hospitalization. It has been estimated that infections due to multidrug-resistant bacteria (about 386,000 in 2007) result in more than 2.5 million extra hospital days and cause 25,100 deaths each year in the European Union, Iceland, and Norway. As a comparison, each year about 48,000 people are killed in road accidents in the same area (EARSS, 2007; ECDC/EMEA, 2009). Infections by antibiotic-resistant strains are associated with a reduced quality of life, with metastatic bacterial infections, an increase in recurrence rates, chronicity, and future opportunistic infections with resistant organisms.

Treatment failure multiplies the risk of complications. Most vulnerable are those with weakened immune systems, such as cancer patients, malnourished children, and HIV-positive people, for whom adequate therapy to prevent and treat severe infections is often essential for their survival. In addition, antibiotic resistance jeopardizes advanced medical procedures such as organ transplants and prosthesis implants, where antibiotics are crucial for patient safety and to avoid complications (Cars and Nordberg, 2004).

The failure of the initial antibiotic regimen underscores the clinical dilemma of using empirical therapy, in the absence of rapid diagnostic tests. However, empirical therapy is difficult in such infections, since it is increasingly difficult to predict resistance. A study in intensive care units demonstrated significantly higher mortality among patients that received inadequate empirical therapy, compared with those given adequate therapy (42% vs 17%) (Kollef et al., 1999). Understandably, there is a clear justification for initial broad-spectrum therapy in severe infections. This creates a vicious circle, because the more the broad-spectrum antibiotics are used, the higher the risk of selecting resistant bacteria (Patterson and Rice, 2003).

Owing to inadequate or delayed treatment, longer periods of infectivity occur in these infections, increasing the number of infected people circulating in the community and thus exposing the general population to the risk of contracting a resistant strain of infection (WHO, 2002).

As indicated, treatment failures in these infections are associated with a greater likelihood of death. For example, in the case of bloodstream infections from MRSA, mortality has been shown to be two or three times higher than in infections with non-resistant strains (Cosgrove et al., 2003). Increasingly complicated diseases and higher mortality has been also shown for resistant *Salmonella* (Helms et al., 2004), *Campylobacter* (Helms et al., 2005) and for vancomycin-resistant enterococci (Edmond et al., 1996). A series of registry-based studies in Denmark determined the mortality associated with gastrointestinal infections by *Salmonella* Typhimurium between 1995 and

1999. Twenty eight of 953 patients infected with pan-susceptible strains died, having a mortality rate 2.3 times higher than that of the general population, whereas infection with multi-resistant (ampicillin, chloramphenicol, streptomycin, sulphonamides, tetracycline, and nalidixic acid) strains (40 patients; 5 deaths) were associated with a mortality rate 13.1 times higher than that of the general Danish population (Mølbak, 2004).

In recent years, links between antibiotic resistance and virulence have been found. Virulence genes, for example those associated with enterotoxin production or iron sequestration, which increase the invasive ability of their host strains, and antibiotic resistance genes can be co-localized in the same mobile genetic elements of genomic islands, thus transferring and expressing simultaneously (Carlson et al., 2007; Doyle and Erickson, 2006). Genes that have a function in both virulence and antimicrobial resistance have been also observed (e.g., efflux pumps in *Campylobacter*) (EFSA, 2008a). Any enhancement of virulence in pathogenic bacteria can adversely affect the outcome of treatment.

Lastly, it should be pointed out that none of the above-mentioned calculations include any estimate of the costs to be incurred by future generations, which will almost certainly be higher than those currently being experienced. Furthermore, the economic and health costs of resistance, heavy enough in the industrialized world, are often even more severe in developing countries because of the state of their economic, health, and infrastructure systems, resulting in irregular supply and availability of drugs and inappropriate use of antibiotics (Cars and Nordberg, 2004).

GENERATION, DISSEMINATION, AND MECHANISMS OF ANTIBACTERIAL RESISTANCE

Throughout this review, “antimicrobial” will be used as a general term to refer to any compounds, including antibiotics and biocides, which have an inhibitory or lethal effect against microorganisms. The term “antibiotic” will be used to refer to natural, synthetic, or semi-synthetic drugs which at low concentrations exert an action against sensitive microorganisms (selective toxicity). They are used to treat, control, or prevent infectious diseases in humans, animals or plants, and to improve efficiency of feed utilization. “Biocide” will be used as a general term to refer to chemical agents (decontaminants, feed and food preservatives, disinfectants) which are usually broad spectrum, intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism (SCENIHR, 2009).

Emergence of Antibacterial Resistance

Although in some instances induction of resistance mechanisms by the presence of an antibiotic has been described, the development of antimicrobial resistance in bacteria is essentially

a Darwinian process of selection of the fittest (Rosenblatt-Farrell, 2009). Human beings (intestine, skin), animals, foods, surfaces, and environment (e.g., soil or water) share bacterial populations. If a stress factor (e.g., an antimicrobial compound) acts on these populations, every susceptible bacterium will die, but not those that are resistant by chance. By selective pressure, these bacteria will survive and multiply, producing a resistant progeny. After a given period of time, the original susceptible population will have been replaced by the resistant population. When antimicrobials are used incorrectly (e.g., for too short a time, at too low a dose, at inadequate potency) the likelihood that bacteria will adapt and replicate rather than be killed is greatly enhanced (WHO, 2002).

Resistance to antibiotics can be innate (intrinsic), related to the general physiology or anatomy of a microorganism (absence of the target of the antimicrobial agent, poor permeability of cell envelope, production of enzymes that inactivate the antimicrobial, or presence of efflux systems that decrease intracellular antibiotic concentration). Intrinsic resistance is an inherent trait in certain bacterial species (referred to as “insensitive” or “unsusceptible”) and is not affected by use (or misuse) of antibiotics. For example, the outer membrane of Gram-negative bacteria makes them relatively impermeable to hydrophobic compounds such as macrolide antibiotics, thus conferring intrinsic resistance to these drugs. Some bacteria can also use temporary strategies in which different genes are expressed or suppressed in order to enable survival in the presence of antibiotics (stress-response systems) (Rosenblatt-Farrell, 2009). However, this intrinsic form of resistance is not the main source of concern for human and animal health. The vast majority of antimicrobial-resistant organisms have instead emerged as a result of genetic changes, acquired through mutation (vertical evolution) or by the uptake of genetic material by horizontal transfer from other bacterial strains (FVE, 2002). The expression of these genetic changes in the cell results in adjustments to one or more biological mechanisms of the affected bacteria and ultimately determines the specific type of resistance that the bacteria develop, resulting in a myriad of possible biological forms of resistance (Alanis, 2005).

Mutational resistance develops as a result of spontaneous mutation at a locus on the microbial chromosome that controls susceptibility to a given antibiotic. Spontaneous mutations usually lead to changes in an antimicrobial target (e.g., chromosomal changes that result in resistance to quinolones) and are transmissible vertically. Mutations in regulators or regulatory regions can contribute to antimicrobial resistance by leading to the overproduction of either intrinsic resistance determinants, such as efflux pumps (Beinlich et al., 2001), or the target itself (Flensburg and Sköld, 1984). Mutations are relatively rare, occurring at random with a low, but consistent, probability in all bacteria: about 1 per 10^7 to 10^{10} cells (Mulvey and Simor, 2009). The extremely high replication rate of bacteria means that there will be huge increases in the number of resistant bacteria. Of greater concern are gene transfer systems that allow the exchange of extrachromosomal genetic material between bacteria, both vertically and

horizontally. Horizontal gene transfer plays a relevant role in accelerating the spread of antibiotic resistance. It can occur between strains of the same species or between different bacterial species or genera sharing the same ecological niche. This is a very worrying issue in the public and animal health context because this mobility expands the opportunities for resistance determinants to pass from non-pathogenic to pathogenic strains.

Indeed, the scientific community is convinced that there is a “pool of resistance genes” in the normal flora present in humans and animals, foods, surfaces, and the environment. The larger this pool, the greater the probability that these genes will be acquired by those bacteria that cause illness. Genes encoding enzymes that can modify the structure of an antibiotic (e.g., penicillinases) as well as genes leading to target modifications or pump efflux synthesis are commonly transferable. It should be pointed out that the acquisition of new resistance determinants may be beneficial to the bacteria under specific stressful conditions, but may have an environmental cost when no selective pressure is present. However, bacteria can evolve and adapt to reduce this cost, by compensatory mutations or fine regulation of resistance expression. This compensatory evolution allows resistant bacteria to persist even in the absence of antibiotic selection pressure (Kempf and Zeitouni, 2012).

Dissemination of Antibacterial Resistance

Selection of resistant bacteria results in the selection of resistance genes that can now spread and propagate to other bacteria. Bacteria are particularly efficient at enhancing the effects of resistance because of their ability to multiply very rapidly, passing on resistance genes when the bacteria replicate (vertical evolution). As indicated in previous paragraphs, resistant bacteria can also pass on their resistance genes to other bacteria through horizontal transfer mechanisms (conjugation, transduction and transformation). The commonest and most efficient genetic transfer strategy is the exchange of conjugative plasmids (extrachromosomal circles of DNA molecules that replicate independently of the chromosome) via the formation of a proteic hollow tubular structure (named “pilus”) which temporarily connects donor and receptor bacteria and allows the passage of these DNA fragments, always leaving a copy behind, and thereby multiplying antibiotic resistance among successive generations within a bacterial colony. Plasmids can be transmitted both vertically and horizontally. It is estimated that most acquired resistance is plasmid-mediated (Alanis, 2005).

Bacteria may also acquire resistance genes through the spread of transposons or integrons, groups of linked genetic elements. Transposons are specialized fragments of DNA that can carry several resistance genes. They cannot replicate themselves, but can move within the genome, thus facilitating resistance gene migration (e.g., from chromosome to plasmid). Bacterial resistance found at the chromosomal level can be

disseminated horizontally because these resistance genes are normally located in transposons. Integrons can also encode several resistance genes. They cannot move by themselves, but encode mechanisms both to capture new antibiotic resistance genes and to excise them within and from the integron, thereby substantially increasing the horizontal mobility of antimicrobial resistance genes. Integrons are commonly carried in plasmids, but they may also be chromosomally-integrated, as in *Salmonella enterica* serotype Typhimurium DT 104 (SCENIHR, 2009). Insertion sequences and genomic islands are also components of the horizontal gene pool.

Transduction is another form of transmission of bacterial resistance genes and occurs via the use of a “vector,” most often viruses (bacteriophages) capable of infecting bacteria. Transformation is a third mechanism of genetic transfer and takes place when there is direct passage of free DNA from one bacterium (generally dead and broken apart close to the receiving bacterium) to another. The receiving bacteria incorporate the free DNA into their own genome in a stable form (Alanis, 2005).

The probability of horizontal gene transfer varies widely among bacterial groups. Species of *Enterococcus* and *Enterobacteriaceae* have developed highly efficient mechanisms for gene transfer. Thus, a high frequency of exchange between unrelated species (including the exchange of resistant genes to virulent strains) is to be expected in the habitat of these bacteria (human and animal intestine). *Enterococcus* spp. and *Escherichia coli* are normally considered indicators of antibiotic resistance. Medium and low risk of horizontal gene transfer has been identified for *Lactococcus* spp. and *Bacillus* spp., respectively (SCENIHR, 2009).

The issue of horizontal resistance gene transfer is addressed either as a direct hazard or an indirect hazard in the food industry. The direct hazard is the presence in foods of food-borne resistant bacteria, which can be transmitted to people (ingestion, contact), where they can cause infectious illness (EFSA, 2008a; Hald et al., 2007). The indirect hazard to human health is through the horizontal transfer of mobile genetic elements (plasmids, transposons, or integrons carrying genes conferring resistance to antimicrobials) from non-pathogenic (commensal, probiotic, technological) to pathogenic bacteria. The transfer of genetic elements involved in resistance can occur anywhere throughout the food chain: in the environment (e.g., effluents), in food producing animals, on food-industry surfaces, in foods or in the human body (e.g., intestinal tract or skin) (Lester et al., 2006). Usually both direct and indirect hazards act together. Although the relative contribution of each stage in the food chain to this indirect risk has not been elucidated so far, researchers stress the importance of waste-water and the intestinal tract of humans and animals in the transfer of genetic elements involved in antibiotic resistance (Hunter et al., 2008; Li et al., 2010). Thus, it has been proposed that a low level of carriage of resistant strains by humans should be a public health goal, in much the same way as normal blood pressure and a low serum cholesterol level are public health goals (Nijsten et al., 1994).

Mechanisms of Antibacterial Resistance

Antimicrobials fight bacteria through a variety of mechanisms: damaging or inhibiting the synthesis of bacterial cell walls (penicillins), affecting bacterial DNA or RNA (quinolones), proteins (tetracyclines), or metabolic pathways (sulphonamides) (Rosenblatt-Farrell, 2009). Microbial strategies for resisting the effects of antimicrobials include biofilm formation, changes in surface permeability, efflux or enzymatic inactivation of the compound before it reaches its target site, modification or overproduction of the target site, and acquisition of alternative metabolic pathways to those inhibited by the drug (IFT, 2006).

In contrast to antibiotics, biocides have multiple target sites against microbial cells. Thus, the emergence of acquired reduced susceptibility is often mediated by non-specific means causing a decrease in the intracellular concentration of biocides (e.g., permeability changes or efflux pumps), and is unlikely to be caused by the modification of a target site or the bypassing of a metabolic process. Modification of the target site has been described on rare occasions as a mechanism for resistance to biocides (e.g., bacterial resistance to triclosan when used at low concentrations) and does not seem to be widespread among bacteria, although there is a paucity of information on this subject (Gómez-Escalada et al., 2005; SCENIHR, 2009). It is likely that various mechanisms operate synergistically in the case of biocides (EFSA, 2008b).

Biofilms

Most bacteria are associated with natural or abiotic surfaces and grow as biofilm rather than as planktonic cells. Biofilms are communal structures of microorganisms encased in an exopolymeric coat (exopolymers and extracellular enzymes) that form on surfaces and are a prevalent mode of growth for microorganisms in nature (Hall-Stoodley et al., 2004). Bacterial cells in biofilms express properties distinct from planktonic cells, such as an increased resistance to biocides and antibiotics (Capita et al., 2003; Cerf et al., 2010; Kim et al., 2007; Lunestad et al., 2007; Shi and Zhu, 2009; Smith and Hunter, 2008; Stickler and Jones, 2008; Williams and Stickler, 2008) through various mechanisms (Table 3).

Table 3 Mechanisms associated with increased resistance to biocides and antibiotics in bacterial biofilms (Højby et al., 2010; IFT, 2006; Lewis, 2008; Pan et al., 2006; Shi and Zhu, 2009; Smith and Hunter, 2008)

-
- Reduced diffusion of the active molecules through the biofilm due to the extracellular polymeric matrix.
 - High concentration of bacteria in the biofilm.
 - Modified physiological state: decreased metabolism and growth rate.
 - The population growing more slowly may be more resistant.
 - Alteration of membrane permeability via decrease of porin synthesis.
 - Induction of multi-drug resistant operons and of efflux pumps.
 - Overproduction of enzymes degrading antimicrobial compounds.
 - Quiescence.
-

Although bacteria within biofilms are more resistant to biocides and antibiotics, the link between the use of biocides against bacterial biofilms and the potential emergence of antibiotic resistance is not straightforward (Jurgens et al., 2008; SCENIHR, 2009).

Permeability Changes

The most common form of resistance to antibiotics is due to the structure and composition of bacterial membranes, which can act as impermeability barriers, either naturally or through acquired resistance mechanisms. This barrier limits the amount of an antimicrobial that enters the cell, thus decreasing the effective antimicrobial concentration. Several reports on reduced biocide efficacy following changes in proteins (Winder et al., 2000), fatty acid composition (Álvarez-Ordóñez et al., 2008; Moorman et al., 2008), phospholipids (Boeris et al., 2007), or lipopolysaccharides (Stickler, 2004) in the bacterial membranes have been published. For example, *E. coli* resistance to beta-lactam antibiotics can occur through changes in outer membrane porins. Resistance to glycopeptides in *Staphylococcus epidermidis* may occur through overproduction of glycopeptide binding sites within the cell wall peptidoglycan (IFT, 2006). Recently, it has been shown that changes in membrane fluidity can play an important role in adaptation of *Listeria monocytogenes* and *Salmonella enterica* to poultry decontaminants (Alonso-Hernando et al., 2010). However, these data are derived from laboratory trials (using increasing sub-inhibitory concentrations of decontaminants) and results still require corroboration in field experiments.

Efflux Pumps

Efflux pumps are transport proteins that actively remove the antimicrobials penetrating the cell, reducing their intracellular concentration to below effective levels. Efflux has been described for several antimicrobials (Alanis, 2005). It may be specific to one compound, or may work on a range of dissimilar compounds, for example, additives and antibiotics (Potenski et al., 2003).

Enzymatic Inactivation

Enzymatic inactivation occurs when the bacteria produce one or more enzymes that modify or degrade the antimicrobial, making it inactive against bacteria. These enzymes are typically specific to a particular antimicrobial or class of antimicrobials. This is a common mechanism of resistance affecting beta-lactam antibiotics via the bacterial production of beta-lactamases, which hydrolyses the beta-lactam ring. Several authors (Cloete, 2003; Demple, 1996; Kümmerle et al., 1996; Valkova et al., 2001) have observed that the enzymatic transformation of biocides is also a resistance mechanism in bacteria, notably to heavy metals (e.g., silver and copper; enzymatic reduction of the cation to the metal), parabens, aldehydes (formaldehyde dehydroge-

nase), and peroxygens (catalase, super oxide dismutase, and alkyl hydroperoxidases, mopping up free radicals) (SCENIHR, 2009).

Target Modification/Overproduction

Target modifications occur when the intracellular receptor of the antimicrobial is modified by the bacteria, for instance, through mutation of ribosomal RNA or other key elements, resulting in the lack of binding and consequently the lack of antibacterial effect. Examples of this mechanism include modifications in the structural make-up of penicillin-binding proteins (PBPs) observed in certain types of penicillin resistance, ribosomal alterations that can render aminoglycosides, macrolides, or tetracyclines inactive, and DNA-gyrase modifications resulting in resistance to fluoroquinolones (Alanis, 2005). On the other hand, the glycopeptide antibiotics act by binding to the terminal D-alanyl-D-alanine of the pentapeptide chain of the growing peptidoglycan polymer. The reduction in susceptibility to these compounds in *Staphylococcus* spp. strains may result from the overproduction of glycopeptide binding sites within the cell-wall peptidoglycan (Sanyal and Greenwood, 1993).

Acquisition of Alternative Metabolic Pathways

Some antibiotics act on enzymes in metabolic pathways. Resistant bacteria can develop a novel metabolic pathway that bypasses the effect of the antimicrobial, so rendering it ineffective. Sulphonamides and trimethoprim interfere with the biosynthesis of folic acid, which is an essential vitamin for bacteria. Sulphonamides compete with *para*-aminobenzoate (PABA) for dihydropteroate synthetase, and thus prevent the production of 7, 8-dihydropteroate (a key step in folate synthesis). Trimethoprim selectively inhibits bacterial dihydrofolate reductase and, in doing so, prevents the reduction of dihydrofolate to tetrahydrofolate (a folic acid derivative). Resistance to sulphonamides and trimethoprim is based on metabolic bypass, due to synthesis of altered dihydropteroate synthase and dihydrofolate reductase, which have reduced susceptibility and affinity for sulphonamides and trimethoprim, respectively (Schmitz et al., 2001).

Many bacteria have become resistant to multiple unrelated antimicrobial classes: multidrug-resistant (MDR) bacteria. The major mechanism of MDR is the presence of pumps which expel a broad spectrum of compounds noxious to the bacterium (including antibiotics and biocides).

ROLE OF THE FOOD INDUSTRY IN THE EMERGENCE OF ANTIBACTERIAL RESISTANCE

It is widely recognized that the “selective pressure” caused by antibiotic consumption, both in human medicine and veterinary husbandry, is the main risk factor for the emergence of antibiotic resistance. Greater use of antibiotics (especially

Table 4 Main antimicrobials used throughout the food chain (EEA, 2010; IFT, 2006; OJEC, 1998a; SCENIHR, 2009)

Antimicrobials: general term used broadly to refer to any compound, including antibiotics, food and feed antimicrobial agents, disinfectants, and other substances, that acts against microorganisms.	
1. Antibiotics: active substances used at low doses to treat infectious disease in humans, animals, or plants, by inhibiting the growth of (bacteriostatic agents), or destroying (bactericidal agents), sensitive bacteria (selective toxicity); such substances may be naturally occurring (e.g., penicillin), semisynthetic (e.g., methicillin), or synthetic (e.g., sulphonamides). Antibiotics are also used in food animals to prevent infectious disease and improve the efficiency of feed utilization and weight gain.	
2. Fungicides: Chemicals used to kill or halt the development of fungi that cause plant disease (e.g., carbendazim, difenoconazole, fludioxonil or thiabendazole).	
3. Biocides: general term that refers to active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism (broad spectrum) by chemical or biological means.	
3.1. Feed preservatives:	substances used with the aim of protecting feed against deterioration caused by microorganisms (e.g., citric acid, lactic acid, sodium benzoate, or sodium sorbate).
3.2. Decontaminants:	biocides applied to surface of fresh produce (mainly red meat, poultry and vegetables) to improve their safety and stability by inactivating or inhibiting growth of pathogenic and spoilage microorganisms (e.g., trisodium phosphate, acidified sodium chlorite, chlorine dioxide, peroxyacids, or lactic acid).
3.3. Food preservatives:	substances used to control pathogens and to prolong the shelf-life of foodstuffs by protecting them against deterioration caused by microorganisms (e.g., nitrites, sulphites, benzoic acid, or lactic acid).
3.4. Disinfectants:	biocides used to improve the microbiological status in food production and processing areas, and in food processing plants. They are commonly applied to air, wastewater, equipment, containers, pipework or surfaces (including food handlers) associated with the production, transport and storage of food or drink (including drinking water). Disinfectants are also used in animal husbandry to: 1) cleaning and disinfection of farm buildings, vehicles and cages used for the transport of animals, 2) creating of barriers (foot dips and tyre-dips located outside animal houses, disinfection of materials during outbreaks of infectious diseases), 3) disinfecting animal surfaces (teat dips or udder cleaning) and 4) preservation of specific products such as eggs or semen. Examples of disinfectants include sodium hypochlorite, quaternary ammonium compounds, ethanol or formaldehyde.

inappropriate use, overuse, and misuse) increases the likelihood of developing resistance (Daikos et al., 2008). Moreover, in recent years the role of the food industry in the increase of antibiotic resistant bacteria has also come under scrutiny, and there is a growing concern over the transmission of resistant bacteria via the food chain.

Antimicrobial Compounds used throughout the Food Production Chain

During food production and manufacturing, a variety of antimicrobials are applied to improve the efficiency of the system, and ensure food quality and safety of the products. Table 4 shows the main antimicrobials used in the food chain. The multiple points throughout the food production chain where antimicrobials are primarily used are shown in Fig. 1. As previously indicated, antimicrobials may create selective pressure that could promote resistance.

Antibiotics

Antibiotics in Animal Production. Antibiotics have been used in food animals for more than 60 years to treat, control, or prevent infectious diseases, and to improve the efficiency of feed utilization and weight gain (Table 5). The use of antibiotics in food animals is essential to maintain a consistent supply of healthy animals entering the food chain. In North America and Europe, an estimated 50% of the tonnage of all antibiotics consumed is used in food-producing animals (WHO, 2002). In 1997, about 11,000 tons of antibiotics were used in the European Union, half for animal husbandry (54 mg/kg

body weight for treatment, metaphylaxis and prophylaxis, and 31 mg/kg body weight as growth promoters) and half for human beings (241 mg/kg body weight) (Ungemach, 2000). Recently, Kools et al. (2008) estimated the use of antibiotics in veterinary medicine in 25 countries in the EU at 5,393 tons, with tetracyclines, beta-lactam antibiotics, and sulphonamides as the most used families. In the United States, approximately 12,650 tons of antibiotics were used in 2007 in veterinary medicine (40% tetracyclines), and about 13% of the total amount of antimicrobials consumed were used as growth promoters (AHI, 2008).

The emergence in the last decade of human pathogens with multiple resistances to antibiotics has focused attention on the veterinary use of these valuable drugs. The World Health Organization has recognized that antibiotic use in animals is likely to have a major impact on the incidence of antibiotic resistance in humans and has published documents acknowledging this problem (WHO, 2004; 2009). Systematic studies have shown that the use of antibiotics in animal husbandry leads to the appearance of new resistance determinants among agricultural bacteria, and to increases in the frequency of these determinants once they have appeared. The principal concern has been that the use of antimicrobials in food animals will select for resistance to these agents in zoonotic intestinal bacteria, and, via food-borne transmission, an infection in human beings will develop that is untreatable. A new variation on this theme has been that commensal bacteria present in human or animal gut might transfer their resistance genes to existing susceptible flora, and thereby set the stage for a non-treatable infection at a later time in the individual's life. Genes involved in resistance to antibiotics which are or have been used exclusively in animals have not only been found in animal isolates, but also in the commensal flora of humans, in zoonotic pathogens like *Salmonella*, and also in strictly human

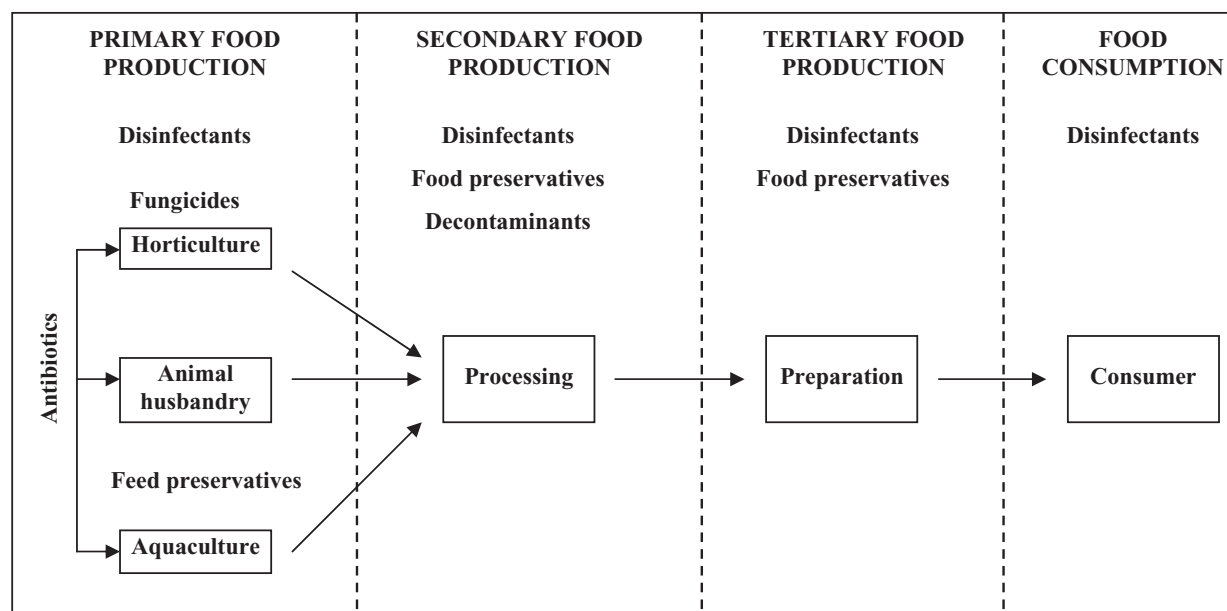


Figure 1 Main antimicrobials used throughout the food chain.

pathogens, such as *Shigella*. This makes it clear that not only the spread of resistant strains, but also the transfer of resistance genes, occurs between animals and human beings (Van den Bogaard and Stobberingh, 2000). The greater the number of resistant bacteria in the intestinal flora, the greater is the likelihood that genes encoding resistance will be transferred to pathogenic bacteria and disseminated into the environment, or from animals to foods of animal origin.

Much evidence supports the view that the inappropriate use of antimicrobials is the critical factor in selecting resistance. In animal husbandry there are numerous examples of inappropriate antibiotic use, for example, the use of antibiotics at sub-therapeutic doses for growth promotion. Flock feeding is another example of inappropriate use. Unlike human medicine, where individual patients can be treated, animal producers must consider medication on a population basis for reasons of animal welfare, logistics, and efficiency, since it is impractical to treat individually each animal in a group which consists of hundreds to tens of thousands. Mass medication is partic-

ularly likely to favor the emergence of, and selection for antibiotic resistance. This issue has been comprehensively addressed elsewhere (WHO, 2009). Furthermore, in primary production, conditions exist which facilitate the spread of bacteria, such as high density of animals. Moreover, antibiotics tend to be given without first diagnosing the cause of the illness. Because treatment is empirical, the antibiotic used may also be one that can be used for a number of illnesses (broad-spectrum antibiotic), rather than one that is targeted towards a specific cause. It has been demonstrated that the broader the spectrum of activity, the higher the chances for bacteria to develop resistance (Alanis, 2005). A fourth example is the policy of allowing veterinarians to sell drugs directly to farmers, thus giving them the financial incentive of maximizing profit.

Moreover, because antibiotics are liberated into the environment as a consequence of their use, a further concern about these compounds is the growing resistance of pathogenic bacteria in the environment. Both human and veterinary antibiotics are often excreted unchanged. The excreted drugs can persist in

Table 5 Application of antibiotics to food animals (IFT, 2006)

- 1) **Treatment.** Administration (preferably with a bacteriological diagnosis) of an antibiotic to an animal or group of animals that manifest clinical disease.
- 2) **Control** (metaphylaxis). Administration of an antibiotic, usually to a herd or flock, in which morbidity and/or mortality have exceeded baseline norms, that is, early in the course of disease onset in the population. In this way infections may be treated before they become clinically visible and the entire treatment period may thereby be shortened. The control concept is based on the premise that due to the risk of infectious disease spreading from an individual animal or small group of diseased animals to the large susceptible population, it is appropriate that all animals be medicated. In addition, modern production systems can mean that often, the only way to treat large flocks is with water medication. For both treatment and control of diseases, antibiotics are applied at therapeutic doses, and must be obtained from or dispensed by, a veterinarian.
- 3) **Prevention** (prophylaxis). Administration of an antibiotic to exposed at-risk healthy animals prior to the onset of a disease for which no etiological agent has been cultured. For example, intramammary infusion of antibiotics to dairy cows at the end of the lactation cycle to prevent mastitis at parturition. The use of antimicrobial agents can be a sign of management problems, and in many countries it is illegal or considered imprudent.
- 4) **Growth promotion.** Administration at low levels (sub-therapeutic doses) for an extended time period to improve the ability of the animal to convert feed into body mass (promote growth rate and feed efficiency) in healthy livestock, primarily cattle, swine, and chickens. Growth promoters (also named feed savers or performance enhancers) are administered directly to animals without direct veterinary involvement.

the environment, creating an opportunity for resistance selection within exposed bacterial populations (Rosenblatt-Farrell, 2009). Thus, it has been observed that the bacterial flora in flies collected from the areas surrounding a poultry production facility demonstrated resistance consistent with the types of antibiotics being used there (Graham et al., 2009).

Although some antibiotic-resistant bacteria inevitably make their way through the food chain and reach consumers (e.g., multidrug-resistant [MDR] *Salmonella* Newport), the magnitude and clinical effect of such an event remains unclear (EFSA, 2008a; Varma et al., 2006). It has been suggested that the overall contribution of animal sources to antibiotic resistance in humans is about 5% (Bywater and Casewell, 2000). Moreover, human zoonotic infections (e.g., *Salmonella* and *Campylobacter*) are only a minor indication for antibiotic therapy, and the overall impact of the use of antibiotics in animals on resistance in human clinical infections seems to be minimal.

Some argue that the impact of antibiotic use in animals pales in comparison with human use, and that efforts should concentrate on the misuse of antibiotics in people. Others warn of the dangers of unregulated and unnecessary use of antibiotics, especially growth promoters, in animal husbandry.

Therapeutic antibiotic use. Antibiotics are used in the treatment and control of many types of infections in a wide variety of animal species. This use can lead to the selection of antibiotic-resistant forms of microorganisms, which is a natural and unavoidable phenomenon. Because most antimicrobials are used for both human and animal treatment, the impact of antimicrobial use in animal husbandry on the therapeutic value of these agents for human disease is of great concern. From a public health perspective, the antimicrobial classes of greatest priority for risk management are quinolones, third and fourth generation cephalosporins, and macrolides (WHO, 2007b).

The development of resistance can be minimized, provided that a number of measures are observed (Table 6). Prudent use of antibiotics is an integral part of good veterinary practice, in order to maximize therapeutic effectiveness and minimize toxicity and selection of resistant microorganisms.

Table 6 Considerations for minimizing the development of bacterial resistance in veterinary medicine (FVE, 2002)

1. **Choice of the right antibiotic:** accurate diagnosis, knowledge of the products approved for the species and indication, the efficacy established in reliable field trials, the predictable sensitivities of possible involved microorganisms, the pharmacokinetics/tissue distribution, immunocompetence status, the spectrum of activity, and the antibiotic combinations.
2. **Use of the right antibiotic therapy:** take into consideration dosage regimen, duration of treatment, group medication, strategic medication, prescribing, delivering, and record keeping.
3. **Implementation of a coordinated antibiotic susceptibility surveillance system.**
4. **Monitoring of antibiotic usage.**
5. **Develop systematic preventive measures to reduce the need to use antibiotics.**

Guidelines exist for responsible (proper, appropriate, judicious) use of antibiotics in veterinary practice (FVE, 2002; OIE, 2010; WHO, 2000; 2001; 2009).

Apart from the epidemiological and environmental issues included in previous paragraphs, the use of antibiotics in veterinary medicine involves the risk of the presence of residues in food of animal origin. Thus, the EU report from 2007 on results from residue monitoring in food of animal origin (European Commission, 2008) included 0.27% of samples with non-compliant results arising from the presence of antibacterial agents in food commodities. In terms of the number of non-compliant results, antibacterials remain the main problem for meat, milk, and honey. In the 2008 RASFF (Rapid Alert System for Food and Feed) report, 3.4% (107 out of 3,139) information and alert notifications dealt with residues of veterinary medicinal products (especially nitrofurans, chloramphenicol, sulphonamides, and erythromycin) in animal-food products (mainly honey/royal jelly and fishery products) (European Commission, 2009a). The risk of the presence of antibiotic residues in foodstuffs is associated with allergic or toxic reactions in consumers, as well as the emergence of antibiotic-resistant strains in the human gastrointestinal tract. To protect citizens from such risks, the European Union Authorities have launched several legislative actions in the last two decades, including, among others, the development of control programmes for monitoring the presence of pharmacologically active substances in food products of animal origin (OJEC, 1996), the establishment of guidelines for veterinary medicinal products (OJEC, 2001a; OJEU, 2009a) and of maximum residue limits (MRL) for these products in foodstuffs of animal origin (OJEC, 1990), and the creation of the European Medicines Agency (EMA) with the Committee for Veterinary Medicinal Products (OJEU, 2004a). An exhaustive review of European legislation on veterinary-drug residues in foodstuffs of animal origin has recently been published (Companyó et al., 2009).

Growth promoter antibiotics (GPA). The growth-promoter effect of antibiotics was discovered in the 1940s, when it was observed that animals fed dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues showed improved growth (Castanon, 2007). The mechanisms of action of antibiotics as growth promoters are mainly related to interactions with the gastrointestinal microbiota. Thus, direct effects of GPA on the microflora can explain decreased competition for nutrients and reduction in microbial metabolites that depress growth. An additional effect is a reduction in gut size, including thinner intestinal villi and total gut wall, which explains the enhanced nutrient digestibility observed with GPA. Finally, a reduction in opportunistic pathogen and subclinical infections, linked to these compounds, has also been observed (Dibner and Richards, 2005).

The United States Food and Drug Administration approved the use of GPA as animal additives, without veterinary prescription, in 1951, and in the 1960s and 1970s each European state approved its own national regulations concerning

the use of antibiotics in animal feeds. The Council Directive 70/524, as well as later modifications, was aimed at European harmonization of regulations concerning additives in feedstuffs. As a result of the recommendations of the Swann report in 1969, molecules that are used for therapy in humans and/or animals have not been used as GPA by most EU-member states. However, many of the GPA that have been used for years in the European Union were analogues of, and show cross resistance with, therapeutic antibiotics (Van den Bogaard and Stobberingh, 2000).

The risk concerning antibiotic residues in edible tissues and products that can produce allergic or toxic reactions in consumers is known to be negligible, as only antibiotics that are not absorbed in the digestive tract are authorized as growth-promoters (Donoghue, 2003). However, the development of antimicrobial resistance and the transference of antibiotic-resistance genes from animal to human microbiota, have been cause for concern for years. The scientific evidence submitted by Scandinavian countries (mainly Sweden and Finland, where the use in feedstuffs of additives from most groups of antibiotics had been prohibited before they joined the EU in 1995), as well as the conclusions of the World Health Organization (WHO, 1997) and the Economic and Social Committee of the European Union (1998) have led to a progressive ban on GPA in the EU.

In the 1990s, it was shown that the GPA avoparcin, a member of the same glycopeptide family as vancomycin, leads to the selection in animals of *Enterococcus faecium* resistant not only to avoparcin, but also to vancomycin. This fact led to a great concern because avoparcin use could create an animal reservoir of vancomycin-resistant enterococci (VRE), representing a potential risk to public health, since VRE have become a major nosocomial infection (WHO, 2003) and can also be transmitted by contaminated foodstuffs (ECDC/EFSA/EMA/SCENIHR, 2009; Peters et al., 2003). In countries where avoparcin has never been used (e.g., Sweden and the United States), no high levels of VRE (*vanA* resistance) has been found in fecal samples of food animals or healthy humans outside hospitals (Bager et al., 1997; Coque et al., 1996). The argument against avoparcin was that its continued non-essential use in agriculture could spread resistance to vancomycin in humans. Avoparcin was, therefore, removed from the European Union market as a precautionary measure aimed at preserving vancomycin's clinical utility (OJEC, 1997).

In July and September 1999, other individual growth promoters were banned by the EU Authorities because they belonged to a class of antimicrobials also used in humans: bacitracin zinc (a polypeptide), spiramycin and tylosin phosphate (macrolides), and virginiamycin (a streptogramin combination) (OJEC, 1998b), or were considered to represent unacceptable occupational toxicity risks (olaquinox and carbadox) (OJEC, 1998c). In 2006, on the basis of the "Precautionary Principle," the four remaining antibiotics used for growth promotion in the EU were banned (OJEU, 2003a): avil-

amycin (oligosaccharide), flavophospholipol (phosphoglycolipid), monensin, and salinomycin (polyethers). Since January 2006, only coccidiostats and histomonostats are permitted as feed additives in the EU. This withdrawal was precautionary, based on the potential for creating a reservoir in food animals of an antibiotic-resistant bacteria population (primarily enterococci) that could be transferred to humans. Non-antimicrobial substances, including enzymes, probiotics, prebiotics, or acids, have been studied in recent years as alternatives for replacing GPA (Castanon, 2007; Rosen, 2004).

It is difficult to assess at present the consequences of the growth promoter antibiotics ban in the European Union. In Nordic countries, where these compounds were banned several years ago, the withdrawal of GPA does not seem to have achieved the desired effect of decreasing the prevalence of human infections by antibiotic-resistant bacteria. Although contradictory conclusions surround the effects of bans, most authors point out that these bans have led to an increase in the use of therapeutic antimicrobials, which might have adverse consequences for animal health and welfare and also financial repercussions for farmers (Casewell et al., 2003; Phillips, 2007; Wierup, 2001a). The main effects of the ban on growth-promoting antibiotics in the Nordic countries are indicated below.

1. Consequences for consumption of antibiotics by food animals.

The ban has resulted in a decrease in the total consumption of antibiotics in animal husbandry, and the complete removal of growth-promoting antibiotics (Wierup, 2001b). For example, in Denmark, where over 105 metric tonnes of antibiotics were used for growth promotion in 1996, use fell to nil by 2000 (Casewell et al., 2003). However, there is concern that banning antibiotics as growth promoters in the European Union might lead to an increase in their therapeutic use, as seems to be happening in Scandinavia. Thus, after the withdrawal of these antibiotics in Denmark (in 1994), animal welfare has suffered, and despite efforts to improve other aspects of husbandry, animal infections have increased, leading to an increase in the use of therapeutic antibiotics (Muirhead, 2002), with an overall increase from 48 tonnes in 1994 to 94 tonnes in 2001 (Casewell et al., 2003). It is clear that the increase in the veterinary use of therapeutic antibiotics (e.g., tetracyclines), most of them identical to those employed in human medicine, could constitute a potential hazard to human health, especially in relation to resistance in zoonotic bacteria (e.g., *Salmonella*). However, it is far from clear that this will apply to the whole of Europe, where farming conditions are different from those in Scandinavia. On the other hand, the impact of this increase in antibiotic consumption is subject to debate, and some authors have reported a minimal (5%) increase in consumption of therapeutic antibiotics following the ban on GPA in Nordic countries (Dibner and Richards, 2005; Wierup, 2001b). Other studies (Grave et al., 2006; Hammerum

et al., 2007) maintain that the former conclusions are the result of misinterpretations of epidemiological data and that in most cases, the initial increase in the therapeutic prescription of antibiotics declined after two or three years.

2. Consequences for animal health and productivity.

The ban was accompanied by a diminution of several glycopeptide resistances among enterococci (considered as an “indicator” species) in food-animal faeces (Casewell et al., 2003; Van den Bogaard and Stobberingh, 2000). For example, in Denmark, before the ban on avoparcin as a GPA, 80% of Danish broilers had vancomycin-resistant enterococci (VRE), while in 2001, after the ban, VRE levels were down to 3%. The prevalence of VRE also decreased in food products of animal origin, in fecal samples from pet animals, and in healthy humans (Singer et al., 2003). On the other hand, Klein et al. (1998) observed similar low percentages of VRE in foods of animal origin before (from 0% to 2.22% of the samples analyzed) and a few months after (from 0.5% to 8.3%) the ban on avoparcin as a GPA in Germany. According to these findings, the above-mentioned authors suggest that the use of avoparcin may not have been responsible for the development of vancomycin resistance in animals. It has been suggested, however, that more than five years are necessary in order to reduce resistance rates in animals in any substantial way (Singer et al., 2003). Reducing the resistance pool in animal faecal bacteria was achieved at the cost of deterioration in animal welfare: loss in production, increased morbidity and mortality, and decreased weight gain (Casewell et al., 2003). Recently, an economic model was developed to assess the impact of a growth-promoter ban in US pork production, and the experience in Denmark was used as the basis for the model. The model predicted that a ban in the US would increase costs by approximately \$4.50 per animal in the first year (Miller et al., 2006). Other authors have reported very little change in food production following the GPA ban, with mortality rates almost unaffected and feed conversion only mildly reduced (Dibner and Richards, 2005; Singer et al., 2003). The ban on growth promoters necessarily requires an improvement in farm hygiene, and it has been shown that under appropriate production conditions, it is possible to attain good, competitive production results without the continuous use of antibiotics in feeds (Castanon, 2007).

3. Consequences for human infections.

An effect on humans attributable to the GPA ban has been some diminution in vancomycin-resistance in *Enterococcus* spp. isolated from human fecal carriers. However, there has been no reduction in the prevalence of resistant enterococcal infection in humans, probably related to the increased use of vancomycin for treatment of methicillin-resistant staphylococci (Singer et al., 2003). Some authors have observed that enterococci isolated from clinical samples are not genetically related to those isolated from meat, and both groups of strains exhibit different resistance patterns, suggesting that foods of animal origin cannot be considered the main source of untreatable

nosocomial infections in humans (Klein et al., 1998). The antibiotic susceptibility of *Salmonella* and *Campylobacter*, responsible for the major zoonoses in Europe (EFSA, 2010a), has not been substantially affected by the ban on GPA because of their spectrum of activity (GPA are mainly active against Gram-positive bacteria). In contrast, increased antibiotic resistance in these zoonotic agents has been observed in Denmark (Casewell et al., 2003) and might be expected in Europe in response to the increased use of therapeutic antibiotics in animals. Moreover, the prevalence of *Salmonella* and *Campylobacter* in animal foods has increased since the ban. The variation in the size of food animals not given growth promoters leads to more frequent rupture of the gastrointestinal tract at slaughter, fecal spillage, and potential contamination with both pathogens (Bremner and Johnston, 1996).

4. Consequences for international trade.

The ban on antibiotics in animal feeds will have consequences for the international meat trade, because the European Union imports foods only if they are obtained from animals that are not fed antibiotics, in application of the Precaution Principle allowed by the World Trade Organization (Castanon, 2007). There has been relatively little regulatory activity regarding GPA use in the United States, where these compounds continue to be authorized under FDA regulations and are controlled on a case-by-case basis (Viola and DeVincent, 2006). It is clear, however, that the practice of using GPA in general is under scrutiny in the US (Angulo, 2004) and that consumer pressure is persuading commerce to remove GPA from animal feeds. For example, some large restaurant corporations (McDonald's or KFC) have developed antibiotic-use policies that exclude human-use antibiotic classes for growth promotion purposes in the flocks and herds of suppliers from whom they purchase poultry and beef products (Dibner and Richards, 2005). Moreover, producers that seek export markets will be forced to give up GPA if they are to sell to the EU and many other markets.

Antibiotics in Aquaculture. Global production of aquatic species (finfish, crustaceans, molluscs, and others) has grown substantially, increasing from 10 million to 50 million tons in the last decade, providing at present more than 40% of the seafood supplied to consumers (Cole et al., 2009). Increase in both demand and production capability has led to increased concern about diseases in aquatic species, including infectious diseases. Estimates for some European countries show that the amount of antimicrobials used per tonne of aquaculture products varies from 2 g (Norway) to more than 40 g to 100 g (Denmark, France, and Greece). Outside the European Union, figures as high as 200 g (Chile) or 700 g (Vietnam) per ton have been recorded (WHO, 2006).

An antimicrobial agent has never been developed specifically for aquaculture applications. The antibiotics used in aquaculture are, therefore, those that have been developed for, and are used

to treat, humans or land-based animals. Those most commonly licensed would be oxytetracycline, trimethoprim-sulphadiazine (or ormetoprim/sulphadimethoxine in American countries), oxolinic acid and/or flumequine, and florphenicol (WHO, 2006). Both in the EU and the US, antibiotics are approved solely to treat diseases as labelled and cannot be used in aquaculture prophylactically or for growth promotion. Antibiotics are incorporated into medicated feeds and are never added to the water to treat bacterial diseases.

The significance for food safety and human health of the use of antibiotics in domestic aquaculture includes: 1) the development and spread of antimicrobial resistant bacteria, 2) the spread of resistant genes, and 3) the occurrence of antimicrobial residues in aquaculture products. Resistant bacteria can cause infections in humans through consumption of contaminated aquaculture food products or through drinking water, and by direct contact with water, aquatic organisms or aquaculture food products. However, most fish pathogens do not infect humans because they are incapable of growing at human and terrestrial animal body temperatures, this fact making the risk of transmission of pathogens from fish to humans apparently small (IFT, 2006).

The development and spread of antimicrobial resistance genes into human pathogens as a consequence of exposure to antibiotics in aquaculture is widely documented. For example, multi-resistance plasmids have been shown to be transferable to *Escherichia coli* from *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Citrobacter freundii*, *Photobacterium damsela* subsp. *piscicida*, *Vibrio anguillarum*, and *Vibrio salmonicida* (Sørum, 2006).

Use of antimicrobials in aquaculture for the treatment of bacterial diseases can result in antimicrobial residues in the food product. According to the RASFF report from 2008, 59% of notifications of residues of veterinary medical products were associated with crustaceans (55%, involving chloramphenicol and nitrofurantoin) and fish (4%, malachite green) (European Commission, 2009a).

Antibiotics in Plant Agriculture. It is generally agreed that the magnitude of antibiotic use in plants is small compared to the extent of use in humans and animal husbandry. In the European Union, there are no antibiotics authorized for use in cultivating plants (European Commission, 2009b). In the United States, streptomycin and oxytetracycline have been used for decades as preventive treatments to control bacteria affecting fruit and vegetables, mainly *Erwinia amylovora*, the causal agent of fire blight, and *Pseudomonas syringae*, the causal agent of apple blister spot. Fruit trees (primarily three species: apple, pear, and peach) account for most of the use of antibiotics on plants in the United States. Trees are sprayed during blossom time, and the edible fruit is not sprayed. It is estimated that less than 0.1% to 0.5% of total antibiotic use in the United States is used for the treatment of bacterial disease when cultivating plants (McManus et al., 2002; Vidaver, 2002). Gentamicin has been used for years in Mexico and Central America for fire blight control. However, this is a critical tool in human medicine for

the treatment of various infections, and it has been withdrawn from use in the United States.

So far, antibiotic residues in plants have not been considered to be of concern with respect to antimicrobial resistance (Vidaver, 2002). However, antimicrobial resistance among plant pathogens raises questions about the potential for compromising the use of antimicrobials in human disease treatment. The greatest concern is that spraying antimicrobials in the open environment and over physically large expanses of land might increase the frequency of resistant genes. Thus, resistance to antimicrobial agents, especially streptomycin, has been found in several bacteria pathogenic to plants (McManus, 2000). It should be pointed out, however, that, as yet, there are no data that indicate transfer of antibiotic resistance determinants from plant pathogens to bacteria causing human disease, under natural conditions (McManus et al., 2002).

Fungicides

Most antimicrobials used in plant agriculture are fungicides. At present, there are no cross-over chemicals with those used in human medicine to treat severe systemic mycoses. However, although the formulations differ in their imidazole or triazole ring or in the side chain, in all cases the fungal target site (the enzyme lanosterol 14 α -demethylase) is the same. Thus, fungicide resistance in plant pathogens may be of concern in medical mycoses (Hof, 2001; IFT, 2006).

In the European Union, residues of antimicrobials on fruits and vegetables are monitored by the European Food Safety Authority (OJEU, 2005a). The 2007 Annual Report on Pesticide Residues (EFSA, 2009b) included 27 EU member states, Norway and Iceland. The total number of samples analysed was 74,305 (fruit and vegetables, cereals, baby food samples, and samples of processed commodities). A total of 3.99% of the samples exceeded the legal limits, particularly in the fruit and vegetable group and processed foods. Fungicides (including thiabendazole and tebuconazole) were the most frequently found pesticides in samples of fruits and vegetables.

With regard to the RASFF report of 2008, 178 notifications (5.6% of total) relating to pesticide residues were included (European Commission, 2009a), with fruit and vegetables being the food products involved. However, acaricides and insecticides represented the greatest portion of notifications.

Biocides

Recent scientific evidence suggests that the selective pressure exerted by the use of biocides, including compounds widely used in the food industry, could contribute to the expression and dissemination of antibiotic resistance mechanisms, both in human and in environmental bacteria. It seems reasonable to assume this possibility, since research indicates that biocides and antibiotics may have some similar and common interactions and target sites with bacteria, and both types of compounds share resistance mechanisms (Sheldon, 2005).

However, bacterial resistance to different types of biocides has been studied and characterized only recently, and only limited scientific research, focusing on specific molecules and specific bacteria, is available to weigh correctly the risks of antibiotic resistance induced by biocide use. Moreover, most of these studies have been carried out in vitro, and evidence in practice is lacking. Wide variations in methodology, as well as different results, are found in the various reports, and some controversies surround the use of biocides and the possibility that their indiscriminate use might reduce biocide effectiveness and alter susceptibilities towards antibiotics.

In view of the large and increasing use of biocides (general available figures show that the consumption of biocides in the EU increased by 4% to 5% per year in the last decade, with a market of €10 billion to €11 billion in 2006; SCENIHR, 2009), the risk of biocide use leading to the selection and spread of antibiotic resistant bacteria is of increasing concern, and recently several scientific committees in the European Union (European Commission, 2001a; EFSA, 2008a; 2008b; European Commission, 1999, 2006b; SCENIHR, 2009; SCHER-SCENIHR, 2008) have published reports on this topic. These reports raise concerns over the inappropriate use of biocides. However, the scarcity of available data makes it impossible to quantify the impact of use and misuse of biocides in the development, selection, survival, and dissemination of antibiotic-resistant strains, and more studies are necessary to determine which biocides and conditions of use create the highest risk of generating antibiotic resistance.

The use of biocides could select for resistance to antibiotics in four different ways:

- Cross-resistance occurs when an antimicrobial selects (selective pressure) for genes expressing resistance mechanisms common to different antimicrobial groups. Barrier impermeability and efflux pumps are the main mechanisms implicated in cross-resistance between biocides and antibiotics (Thorrold et al., 2007; Tkachenko et al., 2007). Although several authors report interactions between bacterial biofilms and the development of resistance to antibiotics and biocides, very little information is available on the cross-resistance of sessile bacteria to antibiotics and biocides. For example, cross-resistance between additives (e.g., sodium nitrate) and tetracyclines, caused by an increased expression of efflux pumps, has been observed in *Salmonella* strains (Potenski et al., 2003). Cross-protection occurs when adaptation to one antimicrobial modifies the physiological response of the bacterium (e.g., decreasing its growth rate), resulting in a temporary decrease in susceptibility to various unrelated antimicrobials (biocides and/or antibiotics) (Alonso-Hernando et al., 2009a; IFT, 2006; Mah and O'Toole, 2001).
- Co-resistance can occur if an antibacterial selects for a gene encoding resistance to such a compound, and this gene is physically linked to a gene expressing resistance to other antimicrobials. Both genes are included in larger genetic elements (plasmids, transposons, integrons) and are transferred in a single event and expressed jointly in a new bacterial

host. This is, for example, the case for tolerance to quaternary ammonium compounds and beta-lactamic resistance in Gram-positive bacteria (Sidhu et al., 2002).

- The use of a biocide might also indirectly select for clones that are resistant to both biocides and antibiotics, because of the specific characteristics of strains (e.g., structure of the cell wall). Clonal drifts have commonly been observed to be the cause of changes in the overall occurrence of resistance to therapeutic antimicrobials among food-borne pathogens, for example *Salmonella* Typhimurium DT 104 (Doublet et al., 2008). A study performed with *Salmonella* isolates from poultry highlighted a significant relationship between acidified sodium chlorite (ASC) resistance (D values) and antibiotic resistance (Capita, 2007).
- Several stresses, including exposure to antimicrobials, can enhance DNA repair by activating the SOS response in bacteria. SOS response is a widespread regulatory network aimed at addressing DNA damage (e.g., caused by a biocide or an antibiotic) by repairing or bypassing lesions (Erill et al., 2007). It has recently been shown that the SOS response controls the activation of integrons. Integrons are complex DNA fragments capable of integrating and regulating the expression of different antibiotic resistance genes, and thus able to confer multiple antibiotic resistances to bacteria (Guerin et al., 2009).

Feed Preservatives. Antimicrobials can be added to animal feed to reduce the total microbial counts and to control the growth of spoilage microorganisms. In the European Union, feed preservatives are considered “technological additives” (OJEU, 2003a). Before authorization these antimicrobials must undergo a safety evaluation by the European Food Safety Authority (EFSA). Most of the products authorized for this purpose are organic acids added to feed or silage (SCENIHR, 2009).

Decontaminants. The prevalence and levels of pathogens on fresh foods (e.g., meat and vegetables) may be controlled by applying an integrated control strategy to the entire food chain. Provided this strategy is followed, chemical, physical, or biological treatments may be applied to these food products to improve their safety and stability by further inactivating or inhibiting growth of pathogenic and spoilage microorganisms. Article 3(2) of Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for foods of animal origin, provides a legal basis for permitting “the use of a substance other than potable water” to remove surface contamination from products of animal origin, hitherto prohibited for years in Europe (OJEU, 2004b). Because permission for such a use must be preceded by a demonstration that the techniques are safe, taking into account the potential pathogenic microflora involved, various European Scientific Committees examined the possible development of antibiotic resistance linked to four substances used to decontaminate poultry carcasses (trisodium phosphate, acidified sodium chlorite, chlorine dioxide, and peroxyacids), concluding that there is no specific research

on the risk of these treatments triggering bacterial tolerance to these substances or increased resistance to antibiotics (EFSA, 2008b; SCHER/SCENIHR, 2008). In May 2008, the European Commission prepared a proposal for a Council Regulation implementing Regulation (EC) No 853/2004 regarding the use of antimicrobial substances to remove surface contamination from poultry carcasses. However, in the light of previous scientific opinions, on June 16, 2008, the European Parliament adopted a non-binding resolution against the antimicrobial treatment of poultry carcasses. The Precautionary Principle is one of the motives behind this resolution. The Commission proposal was subsequently rejected in the European Union Agriculture and Fisheries Council on December 18, 2008, which voted 26 against and one abstention (UK) (OJEU, 2009b). The Council highlighted the need to continue to collect new data from food-business operators and research programs so that both the efficacy of these substances and the development of antimicrobial resistance, as well as possible environmental impact, could be fully assessed. A guidance note from the EFSA was published in 2010 to provide guidelines for dossiers of applications to be submitted to the European Commission, for authorization of substances to be used for the removal of microbial surface contamination of foods of animal origin (EFSA, 2010b).

Recently, several *Listeria monocytogenes* and *Salmonella enterica* strains were subjected to increasing sub-inhibitory concentrations of five poultry decontaminants (trisodium phosphate, acidified sodium chlorite, citric acid, chlorine dioxide, and peroxyacids) and their minimum inhibitory concentrations (MICs) calculated before and after exposure. Results showed that: 1) progressively increasing decontaminant concentrations resulted in reduced susceptibility of strains, with increases in MIC of chemical compounds (adaptation and cross-adaptation), marked in the case of acidified sodium chlorite (Alonso-Hernando et al., 2009a); 2) resistance to various antibiotics increased in *L. monocytogenes* and *S. enterica* strains after exposure to chemicals (especially acidified sodium chlorite) (Alonso-Hernando et al., 2009b); 3) prior exposure to acidic decontaminants (e.g., citric acid) increased the percentage of survival of *Listeria monocytogenes* exposed to several acid stress (Alonso-Hernando et al., 2009c). These findings have important implications for food safety and the food industry, raising concerns over the application of certain decontaminants, as suggested by other authors (Rajkovic et al., 2009).

Food Preservatives. The function of food preservatives is inhibition of spoilage microorganisms and extension of shelf-life, as well as the control of food-borne bacteria (Davidson and Zivanovic, 2003). Regulation (EC) 1333/2008 governs the use of these antimicrobials in the European Union (OJEU, 2008). According to this Regulation, the EFSA must evaluate the safety of food preservatives prior to their authorization.

Potenski et al. (2003) described mutants of *Salmonella enterica* serotype Enteritidis selected after a single exposure to chlorine or to individual food preservatives (sodium nitrite, sodium benzoate, or acetic acid) showing resistance to multiple

antibiotics (tetracycline, chloramphenicol, nalidixic acid, and ciprofloxacin). These authors suggested that a mutation in the *mar* (multiple antibiotic resistance) operon was responsible for cross-resistance of preservative-selected mutants to antibiotics. This *mar* mutation was associated with an increased expression of efflux pumps. The importance of monitoring the use of antimicrobial agents to ensure that appropriate concentrations are used to inactivate target pathogens was emphasized.

Disinfectants. Disinfectants are used to reduce the level of microorganisms at multiple stages throughout the food chain. They are used for cleaning and disinfection of equipment, surfaces, air, and for wastewater treatment in food production and food processing plants. In animal husbandry their uses also include decontamination in fish farming (e.g., eggs), disinfecting vehicles and cages used for the transport of animals, use in foot-dips and tyre-dips present outside animal enclosures, and direct application to animal surfaces (e.g., teat dips or udder cleaning). Where a medical claim is made, disinfectants should be treated as veterinary medicinal products and should be used only if authorized in accordance with the provisions of Directive 2001/82/EC on veterinary medicinal products (OJEC, 2001a). Directive 98/8/EC governs disinfectants in the European Union (OJEC, 1998a). This directive introduced a transitional period of 10 years, during which time existing active substances had to be reviewed with regard to the safety of their use for human health and the environment. The potential for the development of resistance has not been considered so far during this approval for use.

Biocides (mainly chlorous compounds) are also used in fresh water intended for human consumption to maintain its microbiological quality (Directive 98/83/EC; OJEC, 1998d). This application is addressed by the Directive 98/8/EC. It has been reported that suboptimal chlorination of water appears to select for multidrug-resistant *Pseudomonas aeruginosa*, an opportunistic pathogen (Rosenblatt-Farrell, 2009).

Published studies provide different perspectives on the emergence of bacterial resistance due to use of disinfectants. Randall et al. (2007) observed that the use of certain types of disinfectants can increase bacterial resistance to various disinfectants and to antibiotics. These authors exposed eight *Salmonella enterica* serotype Typhimurium strains to different farm disinfectants (tar oil phenol, oxidizing compound, aldehyde-based disinfectant or quaternary ammonium compound [QAC]). Results differed depending on the disinfectant and the strain tested. Exposure to oxidizing compounds notably decreased susceptibility to ciprofloxacin and to various disinfectants by inducing the expression of the AcrAB-TolC efflux system. These authors concluded that the potential for this type of compound to drive resistance to ciprofloxacin is a real concern and may provide a selective pressure for the selection and/or maintenance of ciprofloxacin-resistant strains in the farm environment in the absence of ciprofloxacin itself. Other studies show that the use of a disinfectant could increase resistance to various different disinfectants but not to antibiotics. Stable resistance was observed after exposing *Pseudomonas aeruginosa* strains to gradually increasing concentrations of chlorhexidine diacetate (CHX)

(Thomas et al., 2000). There was no cross-resistance in the CHX-resistant strains to any of the antibiotics tested, although some of the cultures were resistant to benzalkonium chloride. Either the presence of an efflux system and/or changes in the membrane permeability constitutes the mechanisms potentially responsible for this cross-resistance between disinfectants, according to the above-mentioned authors. Ledder et al. (2006) exposed forty different strains of bacteria to increasing sub-inhibitory concentrations of triclosan. Exposure decreased triclosan susceptibility (marked in *Escherichia coli*) but did not affect susceptibility to chemically unrelated antimicrobials.

On the other hand, no modifications in bacterial resistance after disinfectant treatment have been reported by other authors. Gradel et al. (2005) observed that the exposure of 286 *Salmonella* isolates from Danish broiler houses to five disinfectants commonly used in poultry premises did not modify MICs of the compounds. Lastly, a recent piece of work (Cottell et al., 2009) showed that triclosan-tolerant *E. coli* strains were significantly more susceptible to aminoglycoside antibiotics. It was proposed that changes in outer membrane, or the loss of plasmids, may be responsible for this relationship between tolerance to triclosan and antibiotic susceptibility. It should be pointed out that the variable results observed in research reports indicate that there is a need for further studies addressing the impact of the use of biocides and the emergence of antibiotic resistance.

In a recent report, the SCENIHR (2009) classified biocides according to their intrinsic potential for generating resistance/tolerance. Some biocides, because of the nature of their interactions with the bacteria, would be more prone to induce resistance/tolerance. This group of high-risk biocides contains the quaternary ammonium compounds, biguanides (i.e., surface active agents), phenolics, and metallic salts. Highly reactive biocides (e.g., oxidizing and alkylating agents) would present a low risk of emergence of bacterial resistance. Resistance to these biocides results mainly from their inappropriate use. Lastly, several other biocides (isothiazolones, anilides, diamidines, inorganic acids and their esters or alcohols) would have to be classified as medium-risk in terms of emergence of bacterial resistance.

As indicated in previous paragraphs, it is reasonable to assume that under certain circumstances, exposure to disinfectants (as well as to other biocides) will trigger antibiotic resistance. The likelihood of this occurring is related primarily to two particular situations (Cerf et al., 2010; SCENIHR, 2009):

- 1) Frequent application of one or more disinfectants at sub-inhibitory concentrations as consequence, for example, of improper use (erroneous concentration) or inappropriate storage of the formulations, is of concern within the antibacterial resistance context. Where disinfectants are used on an "industrial scale" (e.g., when animal houses are cleaned and disinfected), surfaces may not receive optimum levels of the active agent, and the chances of selecting bacteria with increased resistance are greater. The same concerns could also apply to foot dips outside animal enclosures, where the level of the active agent could be diluted by rainfall. Moreover, it

is also common for the dips to contain a range of biological and other materials, which could serve to inactivate the active component, thus increasing the likelihood of selecting resistant bacteria.

- 2) Biocides that are environmentally persistent and can achieve a residual concentration below the minimum inhibitory concentration, act by maintaining a selective pressure which increases the risk of selecting resistant bacteria.

Genetically-Modified Plants and the use of Antibiotic Resistance Markers

The number of countries planting transgenic crops has increased steadily from 6 in 1996, the first year of commercialization, to 25 (15 developing and 10 industrialized countries) in 2008. This growth rate between 1996 and 2008 represents a 74-fold increase, making it the fastest adopted crop technology in agriculture in recent history. In 2008, the global surface of genetically modified (GM) crops reached 125 million hectares (mainly cotton, corn, soybean, and canola), out of a total potential surface area estimated at 315 million hectares. For example, in 2008, 85% of the 35.5 million hectare national maize crop in the US was transgenic (James, 2008; Stein and Rodríguez-Cerezo, 2009; Visarada et al., 2009).

The techniques used for transferring a new gene into a plant are rather inefficient. Thus, in order to find and select the cells that have been successfully transformed, some kind of marker is needed. To produce a genetically modified plant, the gene that will give the plant its new trait (e.g., herbicide tolerance) is coupled with a marker gene. Plant cells are then transformed with both genes simultaneously. The majority of these marker genes work by giving genetically modified cells the ability to break down a poisonous substance (e.g., antibiotics). Plants cells expressing an antibiotic resistance marker gene are thus not harmed by the antibiotic. Treating the cells after the gene transfer with an antibiotic allows only the successfully transformed cells to survive. These cells also possess the gene of interest. Although the marker gene serves no purpose after this procedure, it remains part of the transgenic plant.

This large number of transgenic crops has led to concerns about risks for human health. After the first cultivation of GM plants in the fields, questions soon arose about the risk of horizontal transfer of antibiotic resistance marker genes to soil bacteria with possible consequences for the bacterial community structure and the spread of antibiotic resistance to soil and clinical strains. This may theoretically happen, via transformation mechanisms, either in fields where GM plants are grown or in the intestinal tract when portions of the GM plant are consumed as food or feed.

Several researchers have been demonstrating for years that gene transfer can occur between different phylogenetic kingdoms (Duggan et al., 2000; Gebhard and Smalla, 1998). The successful transfer of transgenic-borne antibiotic resistance genes to bacteria might be possible according to various scientific

data (Demanèche et al., 2008). This includes long-term DNA persistence in soil, the heterogeneous soil structure favoring contact between DNA and bacteria, the prokaryotic origin of the plant transgenic sequences that represent a specific risk for a facilitated integration into a bacterial genome (Gebhard and Smalla, 1998), and greenhouse conditions (Kay et al., 2002). However, the detection of such events remains very difficult, and in most studies no cellular or molecular evidence that the gene from transgenic plants was transferred to bacteria has been reported. According to several authors (Gay and Gillespie, 2005; Keese, 2008), while fragments of DNA large enough to contain an antibiotic-resistance gene may survive in the environment, the barriers to transfer, incorporation, and transmission are so substantial that any contribution to antibiotic resistance made by GM plants is dwarfed in comparison to the contribution made by antibiotic prescription in clinical practice. Thus, horizontal gene transfer from GM plants into bacteria has been observed only when facilitated by the existence of DNA sequence homology between the transgene and the DNA of the recipient bacterium. Recovery of plant DNA by naturally occurring bacteria has not been demonstrated, even when bacteria are exposed to DNA naturally released from plant tissues. The key barrier to stable uptake of antibiotic resistance marker genes from GM plants by bacteria is the extent of DNA sequence similarity (EFSA, 2009c).

Moreover, even if such transfer events happen, they apparently have no consequences for the bacterial community structure. This is probably partly due to the very low frequency at which these transfer events occur (generally estimated at a 10^{-9} probability of a transfer per exposure; Nielsen et al., 2001) and partly to the limited efficiency of the research protocols. The absence of apparent consequences is also, and mainly, because these genes are already present in bacterial communities. Thus, bacteria that acquired a gene from the plant would not have a specific selective advantage relative to other resistant bacteria. This may indicate that soil is definitely a reservoir in which all bacteria, including clinical pathogens, can acquire the genetic determinants that would permit them to adapt rapidly to present and future antibiotics (Demanèche et al., 2008). It is thought that the risk posed by antibiotic-resistance genes in GM crops to commensal and clinical bacteria should be considered as almost nil because 1) the frequency of horizontal gene transfer from plants to bacteria is extremely low, if it occurs at all, and 2) because the plethora of genes already present in bacterial communities, the ease with which resistance determinants are exchanged among bacteria, and the constant evolution to which they are subjected limit the impact that a newly acquired, yet identical, gene from a plant can have. Nevertheless, owing to the controversy which has surrounded this risk for years, the use as markers of genes expressing resistance to antibiotics which are used in medical or veterinary treatments has been phased out (Dona and Arvanitoyannis, 2009).

In the European Union, GM organisms are regulated mainly by Directive 2001/18/EC, Regulation (EC) No 1829/2003, and Regulation (EC) No 1830/2003 (OJEC, 2001b; OJEU, 2003b;

2003c). The risks arising from the uses of antibiotic resistance genes as marker genes in GM plants have been the subject of two earlier assessments by the European Food Safety Authority (EFSA, 2004; 2007b). Case-by-case evaluations (based on molecular, biochemical, toxicological, and environmental evidence) of the risk to humans, animals and to the environment are performed by the EFSA Genetically Modified Organisms (GMO) Panel in accordance with the scientific principles expressed by Directive 2001/18/EC of the European Parliament and the Council, and detailed in the regularly updated guidance documents of the EFSA (EFSA, 2006a).

Recently, the EFSA's GMO and Biological Hazard (BIOHAZ) Panels issued a new joint scientific opinion on this topic, reviewing and reinforcing the previous assessment positions, and concluding that, based on the current state of knowledge, adverse effects on human health and the environment resulting from the use of marker genes in genetically modified plants are unlikely. This opinion will probably have significant consequences for the authorization of new GM crops, as well as for the GM plants already on the market. However, this opinion was not unanimous, and two members from the EFSA's BIOHAZ Panel expressed their minority opinions, raising concerns about the adverse effects of antibiotic-resistance marker genes on public health and environment (EFSA, 2009c).

Microorganisms Deliberately Introduced into the Food Chain

Bacteria deliberately added to food products are used as functional additives for human health benefits (probiotics) or as technological additives (Wassenaar and Klein, 2008). Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts (Lee, 2009). They have received increasing attention in recent years and have been shown to be useful to human beings. Technological microorganisms (starter cultures) have been proven useful for food fermentation (Yakabe et al., 2009).

Microorganisms intentionally added to foods and feeds should not have any adverse effect on human and animal health immediately after consumption and/or in the short or long term. In the United States, the Food and Drug Administration (FDA) lists (without regulatory status) microorganisms considered safe for specific uses in food applications (GRAS; Generally Recognised as Safe). In Europe, an approach with a similar concept and purpose to the GRAS system has been included in the working programme of the EFSA. This approach, called "Qualified Presumption of Safety" (QPS), provides a qualified generic approval system to harmonize the safety assessment of microorganisms used throughout the food chain, and is applied to all requests received for pre-marking food safety assessments (Chamba and Jamet, 2008). The evaluation of microorganisms for QPS requires several determinations, including the presence of acquired antibiotic resistance genes (Wassenaar and Klein, 2008). In essence, it is proposed that a safety assessment of a defined taxonomic group (e.g., genus or group of related species)

Table 7 Comparison of assessment schemes for food supplements used in the United States (FDA GRAS system) and the European Union (EFSA QPS system) (Wassenaar and Klein, 2008)

GRAS (<i>Generally Recognized as Safe</i>) guidelines	QPS (<i>Qualified Presumption of Safety</i>) guidelines
Apply to food additives in general	Apply to microorganisms only
Determination of GRAS status by the FDA and/or external experts	Determination of QPS status by the EFSA
Open list ¹	Positive list ²
Based on common use	Based on history of use and adverse effects
Describe specific substance or microorganism	Describe taxonomic unit (e.g., genus, species, or strain)
Case-by-case assessment	General assessment

¹Open list means a list of substances that is not comprehensive. Because the use of a GRAS substance is not subject to pre-market review and approval by the FDA, it is impracticable to list all substances that are used in food on the basis of the GRAS provision. The use of a substance is GRAS because of widespread knowledge among the community of qualified experts, not necessarily because of a listing or other administrative activity. The GRAS notification program provides a voluntary mechanism whereby a person may, at any time, inform FDA of a determination that the use of a substance is GRAS. The submitted notice includes a "GRAS exemption claim" that includes a succinct description of the substance, the applicable conditions for use, and the statutory basis for the GRAS determination (i.e., through scientific procedures or through experience based on common use in food). A GRAS notice also includes information about the identity and properties of the notified substance and a discussion of the notifier's reasons for concluding that the substance is GRAS for its intended use; ²Positive list means a list of compounds explicitly authorized and subject to the specific restrictions laid down. The QPS list is supposed to be annually updated by the EFSA.

can be made independently of any particular pre-market authorization process. If the taxonomic group did not raise safety concerns, or if safety concerns existed but could be defined and excluded (the qualification), the grouping could be granted QPS status. Thereafter any strain of microorganism, the identity of which could be unambiguously established and assigned to a QPS group, would be freed from the need for further safety assessment, other than satisfying any qualifications specified. Those strains failing to satisfy a qualification would be considered hazardous and, in the absence of mitigating circumstances, unfit for this purpose. Microorganisms not considered suitable for QPS would remain subject to a full safety assessment (EFSA, 2007c). Table 7 shows a comparison of the assessment schemes of the FDA (GRAS) and the EFSA (QPS).

With the objective of decreasing the development of resistance, the Scientific Committee on Animal Nutrition adopted Opinions defining the criteria used to assess the presence of antibiotic resistance determinants in microbial feed additives (European Commission, 2001b). The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) revised the Scientific Committee for Animal Nutrition (SCAN) Opinions taking into consideration the increase in available scientific data, and published guidelines for the assessment of bacterial resistance to antibiotics of human or veterinary importance (EFSA, 2008c). The guidelines updated by the FEEDAP Panel in 2008, drawn up to eliminate the possibility that microorganisms introduced into the food chain could carry transmissible

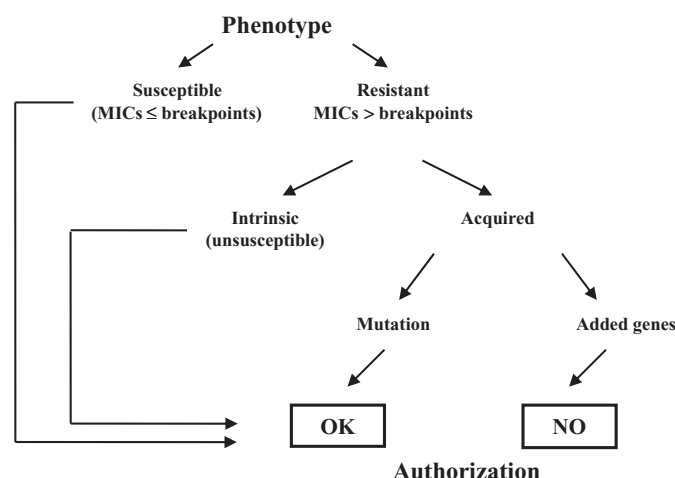


Figure 2 Proposed scheme for the assessment of antimicrobial resistance in a bacterial strain used as feed additive (EFSA, 2008c).

resistances to antimicrobials, covers all the taxonomic groups relevant for the QPS status and could be used as the basis for a qualification. The schema proposed by the FEEDAP Panel for the antimicrobial resistance assessment of a bacterial strain used as a feed additive is shown in Fig. 2.

For the assessment of microorganisms used as feed additives, bacterial strains can be categorized as susceptible (inhibited at the breakpoint level of a specific antimicrobial in a defined phenotypic system; EFSA, 2008c) or resistant. Where all strains within a taxonomic group show phenotypic resistance to an antibiotic, such resistance can be intrinsic to the taxonomic group. Provided that the gene(s) conferring resistance is (are) not associated with mobile genetic elements, the risk of transfer to other microorganisms can be considered as minimal.

When resistance has been acquired by a strain belonging to a taxonomic group naturally susceptible to an antibiotic, the degree of risk of transfer is generally considered to be substantially greater than that associated with intrinsic resistance, except for resistance by mutation of chromosomal genes, which presents a low risk of horizontal dissemination and would generally be acceptable for the FEEDAP Panel. Acquired resistance mediated by added genes is considered as having a high potential for lateral spread (EFSA, 2008c).

The EFSA's BIOHAZ Panel has established lists of microorganisms-granted QPS on the basis of their potential to cause opportunistic infections in humans and to transfer antibiotic resistance genes (EFSA, 2007c; 2008d). Such lists include species in the genus: *Bifidobacterium* sp. (5 species), *Corynebacterium* sp. (1), *Lactobacillus* sp. (33), *Lactococcus* sp. (1), *Leuconostoc* sp. (3), *Pediococcus* sp. (3), *Propionibacterium* sp. (1), *Streptococcus* sp. (1), *Bacillus* sp. (13), *Debaryomyces* sp. (1), *Hanseniaspora* sp. (1), *Kluyveromyces* sp. (1), *Pichia* sp. (1), *Saccharomyces* sp. (4), *Schizosaccharomyces* sp. (1), and *Xanthophyllomyces* sp. (1). The filamentous fungi are not included within the QPS system because they can be used only for specific purposes and because many of these organisms used for food production are known to produce substances of potential concern (mycotoxins).

In spite of their potential usefulness, some microbial groups (e.g., *Enterococcus* spp.) have not been included in QPS lists because of the potential of some species to cause human infections and/or to transfer antibiotic-resistance genes. Moreover, it is frequently very difficult to distinguish pathogenic species from those species which lack known virulence (e.g., the pathogenic species *E. sanguinicola* are indistinguishable from the innocuous *E. italicus*) (Abriouel et al., 2008; Carvalho et al., 2008; Fortina et al., 2008; Gomes et al., 2008; McGowan-Spicer et al., 2008; Valenzuela et al., 2008).

Genetically modified microorganisms (GMMs) are microorganisms in which the genetic material has been altered in a way that does not occur naturally through mating and/or natural recombination. GMMs (bacteria, yeast, and filamentous fungi) can be used in food production either as integral parts in the preparation of various fermented food-stuffs (e.g., lactic acid bacteria for more efficient fermentation or resistant to destructive bacteriophages) or to produce compounds used in food production (additives, amino acids, vitamins, enzymes). The advantages of these microorganisms are that they are easier or faster to grow and that the desired substances are produced in much greater quantities.

The use of GMMs in food products has been slow to materialize in Europe, probably because of consumer reaction to GMMs associated with foods. The production of different compounds probably represents the most common application of GMMs in food and feed production. The potential effects of food-related GMMs in humans are mainly related to interactions with human microflora and gene transfer among and between food-associated and intestinal microbes (Von Wright and Bruce, 2003). According to Regulation (EC) 1829/2003, GM foods and feeds may be authorized for sale only after a scientific assessment of any risks that they might present for human and animal health and, where appropriate, for the environment (OJEU, 2003b). Directive 2009/41/EC lays down requirements for risk assessment of GMMs that must be assessed case-by-case (OJEU, 2009c). The criteria establishing the safety of GMMs for human health and the environment include general criteria (strain verification/authentication, documented and established evidence of safety, and genetic stability) and specific criteria (non-toxicogenic, non-allergenic, non-harmful adventitious agents, criteria related to transfer of genetic material, and safety for the environment). In the European Union, this evaluation is performed by the European Food Safety Authority. A guidance document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use has been published (EFSA, 2006b).

Sub-Lethal Technological Treatments

Contrary to the approach used in traditional preservation systems, which apply bactericidal (lethal) treatments (usually heat treatments), many modern food preservation processes involve

the continuous application of one or more bacteriostatic (sub-lethal) stresses (e.g., pH or osmotic stresses) to slow or prevent bacterial growth in food within the food chain. These technologies are designed to produce safe food, while maintaining its nutritional and sensory qualities (Lado and Yousef, 2002).

After sub-lethal treatments, injured bacteria may act to reduce the impact of stress by making phenotypic and genotypic adaptations (Alonso-Hernando et al., 2009a; 2010). Stress-adapted cells are particularly challenging to the food industry because they may survive processes combining several preservation factors (i.e., hurdle technology). In addition, adaptation is sometimes associated with cross-protection against a range of apparently unrelated challenges, including antibiotics. Examples of these adaptations include expression of specialized protective bacterial proteins (Alekshun and Levy, 1997; Katzif et al. 2003; Lado and Yousef, 2002; Rowan, 1999; Velkov, 1999), induction of the *mar* (multiple antibiotic resistance) operon, which regulates a large number of genes, including those coding efflux pumps (Ma et al., 1995; Rickard et al., 2004), induction of cell repair systems (SOS response) (Aertsen et al., 2004), and generation of genetic variability through random mutations or intra- and intercellular gene transfer (Jolivet-Gougeon et al., 2000; Velkov, 1999). It has been observed in laboratory trials that processing stresses (e.g., high or low temperatures, osmotic and pH stress) can increase horizontal transfer of antibiotic resistance genes by conjugation (McMahon et al., 2007) or transformation (Rodrigo et al., 2010) mechanisms.

It has been suggested that microorganisms are more probably stressed or injured than killed in food processed by non-thermal alternative preservation technologies (mainly high-pressure processing, ionizing radiation, pulsed electric field, and ultraviolet radiation), which could promote the emergence and transfer of antibiotic resistance. However, most reports refer to in vitro studies and the relevance for industrial food processing remains to be defined (EFSA, 2008a; Lado and Yousef, 2002).

FOODS AS VEHICLES OF ANTIBIOTIC-RESISTANT BACTERIA

Epidemiology of Antibiotic Resistance in the Food System

The epidemiology of antibiotic resistance as three key components: food-producing animals, pets, and human populations (Fig. 3). Human beings and animals constitute overlapping reservoirs of antibiotic resistance. Much evidence supports the view that the total consumption of antibiotics (in both human and animal populations) is the critical factor in selecting for resistance. As indicated, the food industry could also play a role in the emergence and dissemination of antibiotic resistance (e.g., via the use of biocides). The resistant (both pathogenic and non-pathogenic) bacteria can be transmitted to humans through food, direct contact and through the environmental spread of faecal waste. Transmission via foods is quantitatively the most important mode of transmission of antibiotic-resistant

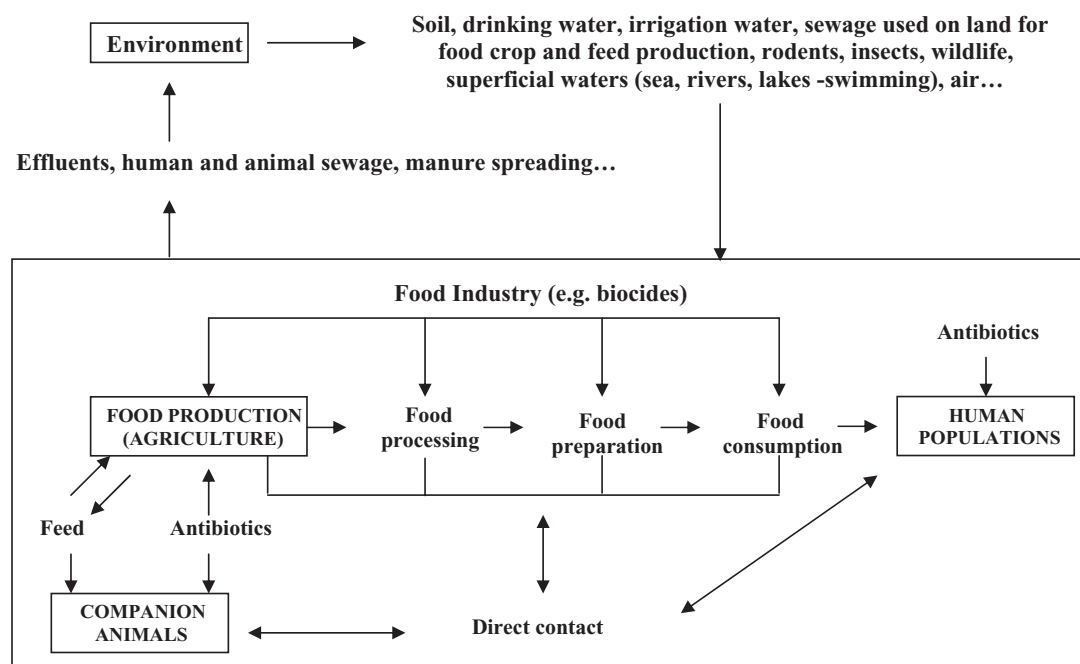


Figure 3 Epidemiology of antibiotic resistance.

bacteria and resistance genes from the farm to the consumer, especially if appropriate standards of food hygiene are not consistently applied throughout the food chain (Van den Bogaard and Stobberingh, 2000). Transfer of antimicrobial-resistant bacteria from animals to humans through direct contact has been shown for vancomycin- and streptogramin-resistant enterococci as well as for methicillin-resistant *Staphylococcus aureus* (European Parliament, 2006; Weese et al., 2006). The resistant bacteria can transfer their resistance genes horizontally to other bacteria at multiple points throughout the ecosystem, as indicated in previous paragraphs. This fact greatly complicates the epidemiology of antibiotic resistance.

Antibiotic-Resistant Bacteria in Foods

In the European Union, Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents obliges Member States to monitor antimicrobial resistance in zoonotic agents and, if they present a threat to the public health, in other agents. This monitoring is mandatory for *Salmonella* and *Campylobacter* in pigs and broilers and products thereof. It should provide comparable data and supplement the monitoring conducted on human isolates. Recommendations regarding the monitoring and reporting of antimicrobial resistance in indicator bacteria such as *E. coli* and enterococci have recently been issued (EFSA, 2008c). The European Food Safety Authority has analyzed and summarized the results, including them in the Annual Community Report on Zoonoses.

Table 8 shows the percentages of sensitive, resistant, and multi-resistant (to more than 4 antimicrobials) isolates from

foods in the European Union according to the most recently published Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Food-borne Outbreaks in the European Union (EFSA, 2007a). The average number of resistances per strain varied between 0.8 (*Campylobacter* isolates from broilers) to 2.0 (*Salmonella* isolates from broilers) and 2.1 (*Salmonella* isolates from pigs).

There are numerous reports directly implicating food-borne antimicrobial-resistant (AMR) pathogenic bacteria in human disease. However, only a limited number of reports have been published confirming transmission of AMR strains from primary production into foods, and subsequently, to the human population. It is very difficult to determine the amount of antibiotic resistance that passes through the food chain, and the role of food in transfer of resistance genes has not been fully explored to date. This is a complex task, as, owing to the delay between food exposure and infection, it will rarely be possible to find a “smoking gun” linking human infections to resistant bacteria acquired from foods proceeding from animals treated with selecting antibiotics. Moreover, there are many possible

Table 8 Percentages of sensitive, resistant and multi-resistant isolates from pig and broiler meats in the European Union in 2006 (EFSA, 2007a)

Microbial group	Number of antibiotics which the strains are resistant to					
	0		1–4		>4	
	Pig	Broiler	Pig	Broiler	Pig	Broiler
<i>Salmonella</i> spp.	11.1%	26.0%	79.8%	63.2%	9.1%	10.8%
<i>Campylobacter</i> spp.	-	7.0%	-	79.8%	-	13.2%

-, not determined.

acquisition routes other than food, such as direct contact with livestock and pets, exposure to the environment, and human-to-human transmission. For example, because the use of antibiotics in humans disturbs the intestinal microflora and decreases the colonization dose considerably, individuals taking an antimicrobial agent are at increased risk of becoming infected with their own intestinal pathogens resistant to that agent. It has been estimated that in the United States resistance to antimicrobial agents results annually in an additional 29,379 *Salmonella* infections, leading to 342 hospitalizations and 12 deaths, and an additional 17,668 *Campylobacter jejuni* infections, leading to 95 hospitalizations (Barza and Travers, 2002).

Molecular genetic studies can provide useful information during outbreak investigations, obtaining isolates derived from animal- or plant-food producers, food and humans, which are then analysed using a discriminatory method and finally compared (Holmberg et al., 1984; Walker et al., 2000). Moreover, using surveillance data, attribution is routinely undertaken in various countries (e.g., Denmark) using mathematical models (Hald et al., 2007).

PREVENTION AND CONTROL OF ANTIBACTERIAL RESISTANCE IN THE FOOD SYSTEM

Despite the fact that it is not yet clear to what extent the food industry contributes to resistance problems in human medicine, it is indisputably a definite factor. Because some microorganisms in human medicine are now being encountered that are so multi- or pan-resistant that it is difficult, and may be soon impossible, to fight them with clinically available antibiotics, every source of resistance must be controlled as strictly as possible in order to protect public health. The need for prevention and control of emergence, selection, and dissemination of resistance should be stressed in the food industry. Studies of antimicrobial use have shown that preventing the emergence of resistance is often much easier than reducing the prevalence of resistance once it has appeared (Singer et al., 2003). The main means for prevention and control of emergence, selection, and dissemination of antibiotic resistance in the food industry are: implementation of standardization, research and surveillance programs, prevention of microbial contamination of foods, and prevention of the emergence or selection of antibiotic-resistant bacteria (EFSA, 2008a; IFT, 2006; SCENIHR, 2009).

Standardization, Research, and Surveillance Programs

Additional information is necessary in order to improve the evidence base relating to biocide resistance and its relation to antibiotic resistance. This information can provide a further means to identify problems, to measure the effects of interventions, and to support policies on the use of antimicrobials. The following are requisites (EFSA, 2008a; SCENIHR, 2009):

1. Design of internationally agreed standard protocols for:

- testing the antimicrobial efficacy of biocides (especially against bacterial biofilms)
- measuring both biocide and antibiotic resistance in bacteria
- evaluating the antimicrobial resistance induced by a selected biocide

2. Increasing research on:

- the identification and characterization, in different fields of application, of the risks of resistance and cross-resistance to antibiotics and biocides ensuing from the use and misuse of biocides. Exposure studies that encompass concentration, environmental conditions (exposure, time, pH, temperature), change in microbial population, and the dissemination of resistant determinants are needed
- the dose-response relationship and the threshold of biocide triggering the emergence of antibiotic resistance and/or the selection of resistant bacteria (no validated methodologies are available). This assessment can take the form of minimal selecting concentration, which is the lowest concentration of a biocide able to select or induce the emergence or expression of a resistance mechanism concerning an antibiotic class in a defined bacterium for a specific duration of exposure
- the role of bacterial biofilms in resistance to both biocides and antibiotics
- the public health relevance of the emergence or selection of antibiotic resistance through the use of biocides (epidemiological studies)

3. Adequately monitoring, by means of well designed surveillance systems, of:

- the current and probable future exposure to biocides and antibiotics (quantitative data): “in use” and residual concentrations, environmental conditions, change in microbial population, dissemination of resistant determinants, and potential synergies or interactions with other molecules
- the prevalence of resistance and cross-resistance to biocides and antibiotics.

Prevention of Microbial Contamination of Foods

The principles applied to the prevention and control of the spread of bacteria via food will also contribute to the prevention and control of the spread of antimicrobial-resistant pathogenic bacteria.

Prevention of Infectious Diseases in Food-Producing Animals and Plants

The health status of food animals and crops can potentially influence food-borne pathogen levels in three ways. First, diseased animals and plants may shed higher levels of food-borne pathogens. Second, animals and plants that require further handling in the processing plant to remove affected parts may lead

to increased microbial contamination and cross-contamination. Finally, certain illnesses may lead to a greater likelihood of mistakes in the processing plant (e.g., gastrointestinal ruptures), which would cause increased microbial contamination and cross-contamination (Singer et al., 2007). Moreover, a reduction in diseases should decrease the need to use antimicrobials in food production.

Prevention of Infectious Diseases in Food-Producing Animals.

Good practice in biosecurity. Biosecurity can be defined as the implementation of measures that reduce the risk of pests and disease agents being introduced and spread. A wide guide to good farming practices for animal production food safety has been published by the World Organisation for Animal Health (OIE, 2006). In the European Union, Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs (OJEU, 2004c) encourages the development and implementation of national and community guides to good practice throughout the food chain, including primary production. This Regulation refers to general hygiene provisions for primary production and associated operations. The commonest biosecurity measures include monitoring and surveillance, prevention, isolation, and elimination. Monitoring and surveillance permit the detection of changes in the prevalence of disease in a given population and its environment as soon as possible. Changes cause alerts, which are often followed by the rapid initiation of specific actions to stop the disease from escalating. Monitoring should be done daily by owners. Global monitoring is arranged by the World Organisation for Animal Health (Office International des Epizooties).

There are a few major principles in disease prevention. Animal buildings should be planned so that animals are raised in batches. Segregation is a very important element in biosecurity: if a pathogen does not enter a holding, no infection can take place. The so-called “all in-all out” system effectively stops the spread of any diseases from one batch to another, and is widely applied in poultry and pig farming. Age segregation of animals is recommended to prevent older animals infecting younger ones. Facilities and vehicles should be adequately cleaned and disinfected between batches. The greatest risk of introducing new diseases into the herd is caused by buying new animals. Animals should proceed only from such farms as can verify their freedom from major diseases. New animals should first be put in quarantine, a check made that they are healthy, and then they can be taken into the herd. Feed and water control is the second most important measure in prevention. Suitable feed (produced locally or in controlled feed mills) and drinking water properly sanitized must be used. Visitors to the farm should be received with caution. Only essential visitors should be allowed, and they should wear farm overalls or disposable clothing. Good management is necessary for prevention of all diseases. In well managed farms animal density is not

too high, general hygiene is good, animal welfare is taken care of and a veterinarian makes frequent, pre-planned visits to the farm, proper identification and registration of animals has been established, and contact between wildlife and farm animals is minimized (Boklund et al., 2004; Tuovinen, 2004).

Once any disease occurs, the isolation of sick animals must be completed as quickly as possible to stop escalation of the problem. Elimination of the disease often means killing the sick animals or all the animals on the farm (Heinonen et al., 2011; OIE, 2006).

Optimal usage of existing vaccines and development of new vaccines. Apart from improving animal health and productivity (increases in daily gain and feed conversion have been reported), veterinary vaccines have a significant impact on public health through reductions in the use of veterinary antimicrobials in food-producing animals. Vaccines may be used to prevent clinical signs of disease after infection or to control, eliminate, or even eradicate an infection at the population level (Meeusen et al., 2007). In the European Union, programs for prevention, detection, and control of *Salmonella* in laying hen flocks of *Gallus gallus* providing eggs intended for human consumption have been implemented. Thus, Commission Regulation (EC) No 1177/2006 states that vaccination programs against *Salmonella* Enteritidis intended to reduce the spread of the disease and the contamination of eggs, must be applied at least during the rearing period to all laying hens at the latest from 1 January 2008 in Member States, as long as they cannot demonstrate a prevalence below 10%. National authorities may exempt a holding from this vaccination requirement provided that satisfactory preventive measures are being applied or that there has been no incidence of *Salmonella* on the holding over the previous 12 months (OJEU, 2006). As a consequence of programs implemented, a major decline in the prevalence of *Salmonella* in laying hen flocks was observed from 2008 and this improved situation may have been reflected in the decrease in cases reported in humans, since eggs are an important source for these infections (EFSA, 2010a).

Interventions on feedstuffs

1. Feed treatments. Feeding is considered a fundamental factor for the health and welfare of animals. The presence of pathogenic bacteria in feedstuffs constitutes a risk for both animal and public health. In order to ensure production of safe feed, HACCP (Hazard Analysis and Critical Control Point) principles and GHP/GMP (Good Hygiene Practices/Good Manufacturing Practices) systems are required to be applied in the European Union throughout the feed-chain, from primary production to animal feeding (OJEU, 2005b). Among the possible means of controlling the contamination of feed with undesirable microorganisms is heat treatment. This is often done as part of the pelleting process to produce a good quality pellet rather than to heat-treat the ration. Moist heat treatment can effectively decontaminate feed materials, as well as compound feed as long as sufficiently high

temperatures and treatment times are used. However, in some circumstances (e.g., pelleted feed for layers) this may not be appropriate. In such cases, a chemical treatment of feed may offer an alternative means of protection. Treatment of feed ingredients or compound feed with blends of organic acids, or with formaldehyde products at suitable concentrations, can be effective in reducing pathogenic microorganisms. Furthermore, chemical treatment has a residual protective effect in feed, which helps reduce recontamination and also helps reduce the contamination of milling and feeding equipment and the general environment (EFSA, 2008e). Moreover, organic acids have been shown to have beneficial effects on performance and some (e.g., butyric) also are effective against pathogenic microorganisms in animal intestinal tracts (Huyghebaert et al., 2011).

2. Competitive enhancement strategies. One method for improving animal health and productivity consists of the utilization of native or artificially introduced microflora populations to reduce pathogens in the intestines of food animals (animal enteric pathogens are an important source for food contamination), by capitalizing on natural microbial competition. Competitive enhancement strategies include probiotics, competitive exclusion (CE) cultures, and prebiotics. Probiotics are live microorganisms which can be included in animal feed, and, when administered in adequate amounts, can help to improve intestinal microbial balance and therefore the natural defences of the animal against pathogenic bacteria. It has been demonstrated that probiotics simulate a non-specific immune response, enhance immune protection, remove pathogenic and conditionally pathogenic bacteria from the organism, and improve assimilation of the nutritive substances (Ross et al., 2010). *Lactobacillus*, *Enterococcus*, *Bacillus*, and *Saccharomyces* are currently the most frequently used probiotics in food-producing animals (Gaggia et al., 2010). Competitive exclusion (CE) technology, also indicated as the “Nurmi concept,” involves the addition of a non-pathogenic bacterial culture of a single strain or multiple strains (typically isolated from the animal species that will be treated), to the intestinal tract of food animals in order to reduce colonization or decrease populations of pathogenic bacteria in the gastrointestinal tract. The introduction of CE bacteria from the intestinal content should occur early in life, such that the CE bacteria are preferentially established in the gastrointestinal system and can become competitive or antagonistic to opportunistic pathogens. CE has been suggested to have several modes of action that eliminate pathogenic bacteria, including: 1) competition for nutrients, 2) competition for physical attachment sites, 3) production of antimicrobial compounds (e.g., volatile fatty acids), 4) enhancement of host immune system activity, and 5) a synergistic interaction of two or more of the above activities (Callaway et al., 2008). Because the use of undefined preparations from intestinal material could result in the transmission of pathogenic microorganisms, regulatory restrictions for probiotic microorganisms have made authorization difficult for this kind of products (Gaggia et al., 2010).

Prebiotics are non-digestible food ingredients that beneficially affect host selectively stimulating the growth and/or activity of one of a limited number of bacteria in the intestinal tract. Probiotics contribute to the establishment of a healthier microbiota that exerts possible health-promoting effects at the expense of more harmful species (Gaggia et al., 2010). Most identified probiotics are carbohydrates and oligosaccharides with different molecular structures. Recent research has indicated that the use of prebiotics, such as inulin and oligofructans, can modulate activity of the immune system directly (Seifert and Watzl, 2007; Swennen et al., 2006). However, prebiotics remain relatively expensive for use with commercially raised animals (Callaway et al., 2008). Symbiotics may be defined as a mixture of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract and induce a synergistic reduction of pathogens and disease (Gaggia et al., 2010).

3. Alternative compounds in feed. Exogenous enzymes (e.g., non-starch polysaccharide-degrading enzymes) in animal feedstuffs have demonstrated their capability to reduce the proliferation of pathogenic bacteria (Huyghebaert et al., 2011). Phytogetic additives (phytobiotics) are substances deriving from medical plants or spices which have a positive effect on production and the health of animals. Whole plants, parts of plants, or essential oils can be used as phytobiotics. Even though the mechanisms of action of these additives are not completely clear, some plant extracts exhibit antibacterial, antiviral, and antioxidant activities (Perić et al., 2009). Orally administered antibodies and cytokines have been proposed as a means of reducing or eliminating pathogens, as well as for improving growth and feed conversion (Joerger, 2002).

4. Bacteriocins, antimicrobial peptides, and bacteriophages. Bacteriocins are proteinaceous compounds that kill or inhibit the growth of bacteria taxonomically related to the producing strain. It has been suggested that bacteriocins administered with feed could influence the ecology of the intestinal microbiota. Such substances have also been proposed as potential animal therapeutics (Joerger, 2002). As with any antimicrobial compound, an issue for concern is the potential of strains to develop resistance and cross-resistance to different bacteriocins. It has been suggested that acquired bacteriocin resistance might decrease antibiotic susceptibility (Naghmouchi et al., 2007). Antimicrobial peptides are small molecules whose production is not confined to bacteria. Development of strains resistant to antimicrobial peptides from previously sensitive strains has been viewed as difficult (Joerger, 2002). However, numerous aspects of resistance to bacteriocins and of the relationship between resistance to bacteriocins and antibiotics remain to be elucidated.

Bacteriophages are viruses that infect and multiply in bacteria. They have unique advantages over antibiotics in that they are both self-replicating and self-limiting. The lytic capability and host specificity of phages present an opportunity for using them to treat infectious diseases in food-producing

animals. Phage therapy has been successfully used for years in Russia, but has failed to gain acceptance worldwide, and it is not yet authorized in most countries (Atterbury, 2009).

The application of feed additives (e.g., probiotics, organic acids, or enzymes) is strictly regulated within the European Union. Their use requires previous evaluation by the European Food Safety Authority (OJEU, 2003a).

Prevention of Infectious Diseases in Food-Producing Plants. Agrochemicals (pesticides) are commonly used, both in fields and in greenhouses, for the control of plant diseases. However, chemical inputs are responsible for several negative effects, that is, the development of pathogen resistance to the agents applied and environmental impacts. Alternative measures for disease prevention and control are included below.

Use of healthy pathogen-free plant propagation material.

All healthy plants are originally grown from pathogen-free seeds. However, numerous systemic diseases are transmitted to progeny via asexually propagated materials infected by microorganisms. The use of pathogen-free planting materials has been proven to be an effective and reliable measure in preventing epidemics of systemic infectious diseases (Dobránszki and Teixeira da Silva, 2010).

Use of disease-resistant or disease-tolerant plant varieties.

The use of resistant varieties is the most effective and economical way to control plant diseases. Resistant and tolerant varieties of various crops have been selected, bred and released to farmers worldwide in recent years (Liu et al., 2007).

Biological control. Applications of antagonistic microorganisms and of beneficial microbes to reduce the incidence of disease in fields are two newly developed technologies. Microbial antagonism implies direct interaction between two microorganisms sharing the same ecological niche. The main mechanisms for the biocontrol of pathogens with antagonistic microorganisms include: a) competition for nutrients and space (e.g., competition for carbon between pathogenic and non-pathogenic *Fusarium oxysporum*); b) inhibition by antibiotics, enzymes, and/or toxic substances (e.g., some *Pseudomonas fluorescens* strains produce compounds responsible for the antagonism expressed against *Gaeumannomyces graminis* var. *tritici* and *Chalara elegans*); c) superparasitism (e.g., the parasitic activity of strains of *Trichoderma* spp. towards pathogens such as *Rhizoctonia solani*); and d) induction of host resistance (e.g., protection of cucumbers against *Colletotrichum orbiculare* after pre-incubation of the cotyledons of the plant with the same pathogen) (Alabouvette et al., 2006). The mechanisms for the biocontrol of pathogens with beneficial microbes include: a) the promotion of plant growth (improved plant vigor) and b) protection against pathogens (competition with pathogens). Beneficial microbes include arbuscular mycorrhizal fungi and plant-growth-promoting bacteria in the root environment (Compant et al., 2005). Only

a few biological control agents are available at present in the European Union.

Application of natural compounds. Inorganic salts (e.g., calcium carbonate), vegetable oils (e.g., menthol), medicinal herb extracts (e.g., powdery mildew), algal extracts (e.g., laminarin), and secondary metabolites produced by microorganisms (e.g., harpin) have been proven to be effective in controlling many plant diseases by killing pathogenic microorganisms and/or stimulating plant defence reactions (Alabouvette et al., 2006; Lin, 2003). Recently it has been suggested that some compounds (e.g., oligochitosan) act by activating innate immunity of plants in a similar way to vaccines (Yin et al., 2010). According to European Regulations, if their intended use is the control of plant diseases they should be considered plant protection products and satisfy the same registration procedures as chemical pesticides.

Adoption of correct hygienic practices in cultivation. Good practices for growing plants are simple and effective ways to reduce the incidence of disease. The main practices are included in Table 9.

Physical control. Solarization (also referred to as solar heating) is a practice that involves passively heating soil covered by a thin clear plastic film for several weeks during the periods of high solar radiation before the crop is planted. This is an effective and current disinfection technique for greenhouse crops in warm and sunny areas in the world and particularly in Mediterranean climates (Scopa et al., 2009). Solarization does not destroy all soil microorganisms, but modifies the microbial balance in favor of beneficial microorganisms, with a consequent reduction in soil-borne diseases (Alabouvette et al., 2006). Disinfection of soil with steam at temperatures from 60°C to 80°C for 3 minutes has proved to be effective against various soil-borne pathogens (Van Loenen et al., 2003). The application of a hot water (40°C) treatment for 2 minutes at 550 MPa has proven to be effective in reducing *E. coli* O157:H7 in alfalfa seeds (Neetoo et al., 2009).

Biofumigation or biodisinfection. A new technique suited to cool regions in the world is biological soil disinfection, which is based on plastic taping of the soil after the incorporation of fresh organic matter. The fermentation of the organic matter in the soil under the plastic results in the production of toxic metabolites and anaerobic conditions, which both contribute to the inactivation or destruction of pathogenic microorganisms (Alabouvette et al., 2006). Lamers et al. (2004) proposed a distinction between biofumigation, which corresponds to the use of specific plant species containing identified toxic molecules, and biodisinfection, which refers to the use of large quantities of organic matter resulting in anaerobic conditions, mainly responsible for the destruction of the pathogens.

Bacteriophages. Phages have been recently evaluated for controlling and preventing the spread of a number of phytopathogens and are now commercially available for some diseases. Major challenges to the agricultural use of phages arise from

Table 9 Correct hygiene practices in cultivation to reduce infectious diseases in plants (Lin, 2003; Alabouvette et al., 2006)

1. Appropriate application of fertilizers to maintain the crop's vigor to resist disease infection.
2. Deep burial of crop residue. This practice helps control certain diseases by placing the organism contained in the residue at a depth where there is an oxygen deficiency. This reduces the population of the disease-causing organism and permits the crop to escape much of the damage.
3. Appropriate water management, that is, regular watering and maintaining appropriate moisture, in order to avoid creation of a suitable environment for the development of waterborne diseases, such as *Phytophthora* diseases.
4. Soil liming to control club root diseases.
5. Rotation of unrelated crops, inclusion of cover crops and appropriate use of fallow (host-free) periods all can contribute to the reduction of inoculum levels for soil-borne pathogens and the increase of diversity in soil microflora.
6. Proper preparation of the field and the subsequent raised beds should reduce plant residues left from previous crops, encourage plant development and reduce problems in areas that are subject to conditions that favour pathogens (e.g., poor drainage).
7. Change of planting/seeding date to escape disease infection.
8. Removal of undesirable plants to aid in preventing infection. Some plants serve as a host reservoir for virus diseases that attack cultivated crops.
9. Removal of diseased plants when they appear. Removal of diseased plants is often an effective method in helping to reduce the spread of a destructive disease.
10. Intercropping with other crops to reduce disease prevalence thanks to slower dissemination speeds.
11. Greenhouses should be equipped with insect-proof nets which prevent the entrance of insects responsible for the spread of viral diseases, and with apparatus to disinfect the recirculating nutrient solution. High-stem crops (e.g., corn) could be used in to form barriers against insects.
12. Use of vinyl or tunnels covered with plastic sheets contributes to protect plants from water-borne pathogens.
13. Bagging of fruits to prevent disease infections and to maintain high quality.
14. Applying other practices such as soil mulching, grassing, and stand cultivation to reduce the severity of soil-borne diseases.

the inherent diversity of target bacteria, the strong probability of the development of resistance, and weak persistence of phages in the plant environment (Balogh et al., 2010).

Improvements to Hygiene Measures throughout the Food Chain

The primary means for preventing the transmission of antibiotic resistance through the food chain is the same as for food-borne pathogens: application of good hygienic practices over the whole the food chain (from the farm to the fork). Good hygiene practices encompass a wide range of measures involved in keeping food safe and wholesome, which can be applied from the growth of food-producing plants and the raising of food animals, through harvesting and slaughter, processing, delivery, storage, final sale, and consumer handling. The Food Hygiene Basic Texts of the *Codex Alimentarius* give a wide-ranging explanation of hygiene practices from primary production through to final consumption, highlighting the key hygiene controls at each stage (FAO/WHO, 1999). In the European Union, Regulation (EC) of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs (OJEU, 2004c) includes general hygiene provisions throughout the food chain. Some important hygienic measures to prevent microbial contamination of foods over the whole the food chain include: 1) correct temperature control, reducing to a minimum the time that foods spend in the danger zone between 5°C and 63°C, cooking raw foods to a temperature that will kill dangerous bacteria, cooling pre-cooked food quickly and storing and displaying high-risk foods at a safe temperature; 2) avoidance of cross-contamination between raw and ready-to-eat foods by direct or indirect contact; 3) the designing of good, easy to maintain food premises and equipment (these should be designed and constructed in such way as to make the maintenance of high standards of cleanliness easy to achieve and to avoid the risk of contamination of food); 4) excellent personal hygiene standards for food handlers (e.g., washing hands frequently and effectively, avoiding bad

habits such as smoking when people are in food areas and using overalls and head coverings when handling foods); 5) notification to manager of symptoms of illness (vomiting, diarrhoea or skin infections); and 6) control of pests by properly trained and experienced pest-control operatives.

Using Fit and Appropriate Technological Treatments

Food processing technologies are designed primarily to reduce the risk of transmission of spoilage and disease-causing microorganisms through the food chain. In recent years, several alternative methods (e.g., mild heat treatments, high pressure processing, pulsed electric field [PEF], or intense light pulses) have arisen to replace historically proven heat treatments, in an attempt to satisfy the demands of consumers relating to food quality. It has been observed that when novel and mild inactivation technologies are applied, incomplete inactivation and sub-lethal damage of microorganisms is often obtained and growth during shelf life of the food product may take place, resulting in a public health hazard (Rajkovic et al., 2010). There is also concern about the fact that the stress imposed through sub-lethal food preservation technologies could change the virulence characteristics of the surviving population and induce cross-resistance to several different stresses, making these organisms more difficult to eliminate in successive treatments, which may, in turn, increase the likelihood of the transmission of bacteria through the food chain (Rajkovic et al., 2009; Rodrigo et al., 2010). A further concern is that sub-lethal food processing treatments might play a role in an increase in antibiotic resistance. Several reports concerning laboratory trials performed suggest that these stresses contribute to the generation, selection, and transfer of antibiotic-resistant bacteria (see section titled "Sub-Lethal technological treatments"). However, the extent to which food-processing technologies contribute to the emergence and dissemination of resistant bacteria in foods

and their practical significance for public health requires further research (EFSA, 2008a).

Proper Management of Manure, Food-Industry Wastewater, and Animal By-Products

Manure (excrement from farm animals) has been associated with numerous zoonotic pathogens, including *Salmonella*, enterohaemorrhagic *E. coli* (EHEC), *Campylobacter*, *Leptospira*, *Yersinia*, *Clostridium perfringens*, *Mycobacterium*, and *Brucella*. Threats from protozoa and viruses are also sometimes mentioned (Cliver, 2009; Venglovsky et al., 2009). Zoonotic microorganisms are likely to contaminate food when manure is used as a soil amendment or when manure contaminates water that is used for irrigation or comes into contact with food (e.g., when used for washing food). Of particular concern is the production of leafy vegetables eaten raw. Although the proportion of food-borne or water-borne disease resulting from contamination with farm animal manure is not well established, several outbreaks from leafy green vegetables contaminated with manure have attracted a great deal of attention in recent years (Cliver, 2009).

The obvious approach to preventing pathogens in manure from contaminating food or water is to eliminate the pathogens from their animal reservoirs. Although considerable progress has been made in the elimination of certain pathogens (e.g., *Salmonella* on poultry and swine farms in Europe; EFSA, 2010a), completely effective means to this end are not yet to hand. Methods of treating manure and biological waste to kill pathogens (disinfection) are available.

In Europe, more than 65% of livestock manure is handled as slurry, a mixture of urine, faeces, water and bedding material (Albihn and Vinnerås, 2007). A common manure management practice is storage. It has been demonstrated that the degree of sanitation that occurs during storage is not sufficient. Some pathogens (e.g., *Salmonella* or EHEC) can persist in slurry for up to several months (the lower the temperature, the longer the survival) and even proliferate substantially if conditions are favorable, for example, as regards nutrient availability, and so the regular transfer of fresh manure from livestock buildings may help sustain microbial populations during storage (Wang et al., 1996).

In Europe, it is also a frequent practice to handle manure in solid form, including feces and bedding with or without excreta in liquid form. This process is known as composting or aerobic digestion. Slurry may also be successfully composted by forced aeration, or following liquid-solid separation using mechanical methods and/or polymer flocculation (Albihn and Vinnerås, 2007). Solid livestock manure is perceived as relatively safe due to the higher temperatures that can develop in this material during storage which contribute to some reduction in pathogen numbers. However, it has been demonstrated that pathogenic bacteria may persist for long periods in animal manure under typical conditions (e.g., survival periods as long as 630 days were observed for *E. coli* O157:H7 in sheep manure;

Kudva et al., 1998). The destruction of bacteria is accelerated by increasing temperature, lowering moisture, and when aeration is used (Venglovsky et al., 2009). Sanitation is achieved if $>50^{\circ}\text{C}$ is reached and this temperature is maintained for a sufficient time, varying from hours to days depending on the organism and the structure of the material. The WHO gives a recommendation of a minimum of a week of treatment above 50°C for the composting of faecal matter (WHO, 2006). Efficient sanitation requires the compost to be repeatedly turned and/or thoroughly mixed because such aeration encourages the action of other microbes that adversely affect the pathogens. Incorporation of structural material may be needed, plus an insulation layer above and below the compost. More technically advanced practices, such as the preheating of incoming air, can also decrease the sub-volumes within compost heaps maintaining temperatures below 50°C (Albihn and Vinnerås, 2007). Pathogenic bacteria may grow in such cold zones, at least as long as sufficient nutrients are available. Stabilization of the treated material, whereby easily degradable organics are degraded, minimizes the risk of pathogen re-growth (Sidhu et al., 2001). The smaller the volume staying at a low temperature, the more efficient the degree of sanitation and thus the fewer turnings of the material needed to reach the sanitation goals. Controlled reactor composting permits the total volume remaining at low temperatures to be decreased compared with open composting, because the reactor walls have some insulation.

Anaerobic digestion of manure and biological waste in general has a long history in Asia. In Europe interest in this treatment is growing in some countries, such as Denmark and Germany. Most farm-scale plants use a continuous process, which is less reliable with regard to sanitation. During mesophilic anaerobic digestion (between 30°C and 38°C) many bacteria and some viruses need more than 2 days for a $1 \log_{10}$ reduction. Thermophilic digestion (at 50°C to 58°C) has proven to be effective in reducing indicator bacteria and *Salmonella* sufficiently (EFSA, 2007d).

Ammonia treatment uses uncharged ammonia for the inactivation of microorganisms. This treatment is efficient for inactivation of bacteria, parasites, and some viruses. The recommended treatment for manure is either 0.5% NH_3 for one week, or 2% urea for two weeks at temperatures above 10°C , or for one month at temperatures below 10°C (Albihn and Vinnerås, 2007). Other chemical and physical disinfection treatments tend to be more costly than the above-mentioned systems (Cliver, 2009).

It should be noted that treatment goals vary in accordance with the origin of the manure (e.g., livestock with or without *Salmonella* infection), and on the potential use of the end-product (e.g., vegetables to be consumed raw or energy crops). According to Albihn and Vinnerås (2007), the treatment effect should be continuously monitored by checking the process (i.e., temperature, pH value, time) and the end-product (microbiological analysis). It should be considered that, despite adequate treatment of manure, a risk for multiplication through post-treatment re-growth exists for some bacteria when complete

elimination is not achieved during treatment, or when introduction occurs while nutrients are still available.

After land application, both the survival and the growth of pathogens vary considerably between different types of microorganisms, with parasites, spore-forming bacteria, and some types of viruses generally persisting for the longest periods of time in the environment (Albihn and Vinnerås, 2007). Microbial survival also varies considerably in response to climate, season, vegetation, soil type, and different environmental factors (Cools et al., 2001; Nicholson et al., 2004). In general, survival is prolonged in a cold climate, a fact that must be taken into consideration if a quarantine period is to be set up as a safety precaution between spread of the end-product and crop harvest or livestock grazing (Venglovsky et al., 2009). Pathogen survival is also favored in aqueous environments, and thus water availability and movement are the most important factors and are affected by moisture retaining properties of soils. The method of application to the land is important too, as ploughing-in or injection reduce bacteria spread and animal exposure. However, persistence may be prolonged within the soil as compared with surface application, which may be related to decreased exposure to UV radiation, temperature extremes, and desiccation, or the increased availability of nutrients (Hutchison et al., 2005). The presence of vegetation may provide a protective environment and enhance survival of bacteria including pathogens (Ogden et al., 2002). Gibbs et al. (1997) observed that some changes in environmental conditions (e.g., rainfall) increase bacterial populations. These authors have reported a survival of pathogens in soil for over a year. An issue for concern is that wild animals may be infected with a pathogen present in the soil and thus acquire a disease or act as a disease reservoir without displaying clinical symptoms (Albihn and Vinnerås, 2007). It should be noted, however, that, in order to define the best management practices, further information is needed on the concentration and survival of microorganisms present in manure and their potential re-growth after treatment or, under favorable circumstances, after spreading.

There is also concern, especially in relation to intensive livestock units, about the presence of antibiotics in animal manure that may pass into the environment, particularly surface water, after the application of these materials to land, and contribute to direct toxicity to environmental microflora and fauna, to antibiotic resistance development, and/or to production of adverse reactions in those with antibiotic allergies. It has been estimated that about two-thirds of the amount of antibiotics consumed are excreted unchanged (Kümmerer et al., 2000).

Determination of the fate of unabsorbed antibiotics excreted in live-stock manure is a relatively new field of study. With only a small number of studies, it is difficult to determine whether antibiotics found in livestock manure may have an effect on human health once they are released into the terrestrial environment. However, data from several studies suggest that with each manure remediation (e.g., composting) the quantity of detectable antibiotics drops significantly because active compounds are degraded. Moreover, with regard to this degradation, most an-

tibiotics become much less stable once applied to soil. As such, the concentrations of most antibiotics found in sources that could have an impact on human health, such as ground or surface water, or plants, are at least an order of a magnitude lower than the concentrations judged to represent an appreciable risk (Venglovsky et al., 2009). However, some drugs can cause problems during biological treatment, since they have the ability to inhibit natural biological processes at relatively low concentrations (Loftin et al., 2005). In addition, some pharmaceutical compounds are very stable in the environment. For example, no break-down was observed for tetracycline during 152 days in a soil microcosm (Jensen et al., 2002). Additional investigations are needed to improve current knowledge about the presence of antibiotic residues in manure and sewage and their impact on the safety of the human food chain.

In respect of the occurrence of antibiotic-resistant bacteria, as previously mentioned, repeated exposure of bacteria to antimicrobial agents and access of bacteria to increasingly large pools of antimicrobial resistance genes in mixed bacterial populations are the primary driving forces for emerging bacterial resistance. Transmissions of antibiotic-resistant genes from manure and sewage bacteria to pathogen bacteria have been achieved in the laboratory. There is, indeed, a potential risk of antibiotic resistance transfer, but the extent of the risk is not well known (Venglovsky et al., 2009). The role of environmental contamination from livestock wastes in promoting antibiotic resistance is difficult to evaluate against the background of the high frequency of resistance to antibiotics found in soil bacteria (DCosta et al., 2006). Nevertheless, prudent use of antibiotics in animal production, particularly of those that are important for both human and animal health, is advised in order to prevent dissemination of antibiotic resistance from manure (McEwen, 2006).

With regard to food-processing industries, they use a significant amount of water and generate wastewater that contains high concentrations of nutrients, organic matter, residual chemicals, and microorganisms (Tarver, 2008). The treatment of food-processing wastewater is compulsory in the European Union (OJEC, 1991). For some food processors, sewage surcharges are supplied to municipal wastewater treatment plants. For other food processors, wastewater translates into investment in either pre-treatment systems before discharging wastewater into publicly owned wastewater systems, or in full-treatment systems that completely treat wastewater before reusing it or discharging it directly into receiving waters. In most treatment plants, wastewater undergoes 2 or 3 different stages of treatment: 1) primary treatment, involving sedimentation (sometimes preceded by screening and grid removal) to remove gross and settleable solids; 2) secondary treatment, involving biological or chemical processes to reduce biochemical oxygen demand (BOD), chemical oxygen demand (COD), and suspended solids (e.g., lagoon systems); and 3) tertiary treatment, involving the removal of a high percentage of suspended solids and/or nutrients (it may include processes such as filtration, coagulation, and flocculation), followed by disinfection. It has been observed that most wastewater treatments are effective

in substantially reducing pathogen loads (García-Armisen and Servais, 2007). However, wastewater treatment plants were found to be reservoirs for antibiotic-resistant bacteria and plasmids involved in resistance, which are released with the final effluents into the environment (Szczepanowski et al., 2004).

Wastewater treatment plants are probably primary routes of entry for antibiotics into the environment. Several studies have also described the occurrence of different antibiotics in both untreated and treated water (Kim and Aga, 2007). The majority of the studies describe lower concentrations in treated water, suggesting a partial removal in wastewater treatment plants. However, it has been indicated that biodegradation does not occur for all antibiotics in these plants (Kümmerer et al., 2004).

Animal by-products (entire bodies or parts of bodies of animals or products of animal origin not intended for human consumption) obtained in food industries (primary exploitations, slaughterhouses, processing plants, or retail outlets) should be collected, transported, stored, handled, processed, and used or disposed of, following the guidelines established to prevent these products from presenting a risk to animal or public health. In the European Union, Regulation (EC) No 1774/2002 (OJEC, 2002) and Regulation (EC) No 1069/2009 (OJEU, 2009d; applicable from March 4, 2011), define types of animal by-products. Category 1 includes, for example, material from ruminants which may be suspected of being infected with, confirmed with, or killed in the eradication of, transmissible spongiform encephalopathy; Category 2 includes, for example, carcasses containing residues of veterinary drugs and certain contaminants; and Category 3 includes, for example, parts of animals not intended for human consumption for commercial reasons. The measures to be implemented for the processing of these by-products are also set out in these Regulations.

Prevention of The Emergence or Selection of Antibiotic-Resistant Bacteria

As antimicrobial resistance in food-borne pathogens and commensals represents a specific public health hazard, additional control measures for antimicrobial resistant bacteria may therefore be necessary.

Appropriate and Prudent use of Disinfectants, as Part of a Good Hygiene Routine

The need for proper use of disinfectants should be stressed and food handlers should be trained to comply with clear and agreed policies and practices, avoiding unnecessary and incorrect use of biocides. Organic debris should be removed before disinfection, and compounds should be applied with regard to proper duration, concentration, pH, temperature, and mode of contact.

An important issue is the choice of the appropriate product on the basis of a risk assessment. The selection of resistant or insusceptible bacteria after exposure to a disinfectant should be considered by national and international risk-assessment bodies. According to the European Commission's independent Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR, 2009), potential factors that should be included in the risk assessment of disinfectants include: a) predisposition of bacterial species to acquire resistance; b) induction of antibiotic resistance genes via genetic cascade; c) type of antimicrobial (intrinsic potential for generating resistance); d) concentration/persistence of molecules present in disinfectants; e) form of growth (planktonic status or biofilms); f) environmental factors; and g) prevalence of bacterial species. In the European Union, additional data are necessary to formulate an appropriate risk assessment (SCENIHR, 2009).

It would be desirable to produce more effective disinfectant formulations. It is important to take into account that European Regulation No 145/2007 and European Decision 2008/809/CE prohibit numerous active substances. The impact of these norms could influence the overall activity of a biocide formulation and should be considered in future risk assessments.

Correct and Reasonable Use of Antibiotics in Agriculture

Reducing of the use of antibiotics reduces selective pressure and in many cases will thereby reduce the prevalence of resistant bacteria. If antibiotics have to be used during the pre-harvest phase, the use of small spectrum molecules should be preferred, and there should be an effective veterinary antibiotic policy. It is important that administration of antibiotics be under the supervision and control of veterinarians. Is also the role of veterinarians to raise awareness and educate farmers in the responsible use of antibiotics and other veterinary drugs. Records for antimicrobial consumption need to be detailed, including information of the animal species, compound and regimen applied (e.g., dose and route of administration), to evaluate compliance with, and the effect of, antibiotic policies (ECDC/EFSA/EMEA, 2009). When farm animals or fish are treated, the whole herd, flock, or shoal will be treated (mass medication) rather than just the infected animal. This fact raises concern because sub-therapeutic doses are received by numerous individuals.

Use of Probiotic/Technological Microorganisms without Genes Capable of Horizontal Transfer

Bacteria added intentionally to the food chain should be free of potentially transferable antimicrobial resistance before their application in feed or food processing. The European Food Safety Authority has published lists of microorganisms to be intentionally added to feed and food formulations on the basis of their potential to cause opportunistic infections and to transfer antibiotic resistance genes (EFSA, 2007c; 2008d).

CONCLUSIONS

The problem of antibiotic resistance, although not new, has greatly increased over the past two decades. It is now an issue for concern in the treatment of infections, and it is likely that the problem will continue in coming decades. Resistance to antibiotics is not eradicable, but will have to be managed. The potential impact of the food system in the emergence of antimicrobial resistance has received increasing attention in recent years. Because much evidence supports the view that the main risk factor for the increase in bacterial resistance is the use (particularly overuse and misuse) of antibiotics, it has been suggested that improving their use in primary food production should be a priority if the emergence and spread of resistance throughout the food chain is to be controlled. Genetically modified organisms (GMO) with marker genes coding for antibiotic resistance, microorganisms added intentionally to foods capable of horizontal exchange of resistance genes, technological treatments used at sub-lethal doses, and the use of antimicrobials (disinfectants, feed and food preservatives, or decontaminants) throughout the food chain comprise the main issues for concern.

Although the relative contribution of the food industry to the emergence of antibiotic resistance remains unclear, the food system should play its part in minimizing resistance whilst continuing to deliver a safe, high-quality food supply. Control of antibiotic-resistant bacteria and their related genes in the food industry requires the prevention of both the emergence and the spread of antimicrobial-resistant bacteria in primary food production, food processing, food preparation, and consumption. Moreover, protocols and methodologies for testing antimicrobial effectiveness and resistance should be standardized, and additional research to understand the scientific basis of the development of resistance throughout the food chain should be carried out. There is also a need for more intense and well designed coordinated monitoring and surveillance programmes in order to ascertain exposure and prevalence of resistance to antimicrobials.

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