Comprehensive Reviews in Food Science and Food Safety

Antimicrobial Resistance: Implications for the Food System

An Expert Report, Funded by the IFT Foundation

ABSTRACT: The safety of food worldwide remains challenged by the potential for emergence of new pathogens and re-emergence of known pathogens. Microorganisms have an inherent ability to evolve—to mutate and adapt to environmental stressors—allowing them to survive otherwise lethal conditions. The Institute of Food Technologists (IFT),[1] the 22000-member nonprofit scientific and educational society, convened a panel of internationally renowned experts to address the concern that the use of antimicrobials in food production, manufacturing, and elsewhere may lead to the emergence of foodborne pathogens that are resistant to antimicrobials, thus compromising the ability to subsequently control them, whether in production agriculture, food processing, or human medicine. The outcome of the panel's deliberations is presented in this Expert Report. IFT's objective for this Expert Report is to increase the understanding—among IFT members, senior policy officials, and other interested groups—of the state of the science on the public health impact of the use of antimicrobials in the food system, and development and control of antimicrobial resistance. This report is the fourth Expert Report produced by IFT.

IFT Expert Report Panelists

IFT is immensely grateful to the panelists for the time and effort that each of them expended in developing and contributing to this Expert Report, bringing their expertise and insight into the state-of-the science on the topics addressed here. Panelists participated in full-day meetings and devoted considerable additional time to report drafting, participating in conference calls to discuss drafts, and reviewing drafts. IFT sincerely appreciates these experts' invaluable dedication to furthering the understanding of antimicrobial resistance.

The participants on the Expert Panel were selected for their scientific expertise. Their contributions represent their individual scientific perspective and do not necessarily represent the perspective of their employer.

Michael P. Doyle, Ph.D.
Panel Chair
Regents Professor and Director, Center for Food Safety
Univ. of Georgia

Francis (Frank) Busta, Ph.D.
Director, The National Center for Food Protection and Defense
Professor Emeritus, Food Microbiology
Emeritus Head of Dept. of Food Science & Nutrition
Univ. of Minnesota

Bruce R. Cords, Ph.D.
Vice President, Food Safety and Public Health
Ecolab, Inc.

P. Michael Davidson, Ph.D.
Professor, Dept. of Food Science & Technology
Univ. of Tennessee

John Hawke, Ph.D.
Associate Professor, Dept. of PBS
Louisiana State Univ.

H. Scott Hurd, D.V.M., Ph.D.
Director
WHO Collaborating Center for Risk Assessment and Hazard Identification in Foods of Animal Origin
Iowa State Univ.

Richard E. Isaacson, Ph.D.
Professor and Chair, Dept. of Veterinary and Biomedical Sciences
Univ. of Minnesota

Karl Matthews, Ph.D.
Associate Professor, Dept. of Food Science
Rutgers, the State Univ. of New Jersey

John Maurer, Ph.D.
Associate Professor, Dept. of Avian Medicine
Univ. of Georgia

Jianghong Meng, Ph.D.
Associate Professor, Dept. of Nutrition & Food Science
Univ. of Maryland

Thomas J. Montville, Ph.D.
Professor, Food Microbiology, Dept. of Food Science
Rutgers, the State Univ. of New Jersey

Thomas R. Shryock, Ph.D.
Senior Microbiology Technical Advisor
Elanco Animal Health

John N. Sofos, Ph.D.
Professor, Dept. of Animal Sciences
Colorado State Univ.

Anne K. Vidaver, Ph.D.
Professor and Head, Dept. of Plant Pathology
Univ. of Nebraska, Lincoln

Lyle Vogel, D.V.M., M.P.H., D.A.C.V.P.M.
Director, Scientific Activities Division
American Veterinary Medical Association

[1] Founded in 1939, the Institute of Food Technologists is a nonprofit scientific and educational society with 22000 members working in food science, technology, and related professions in the food industry, academia, and government. As the society for food science and technology, IFT brings sound science to the public discussion of food issues.

IFT Staff

Jennifer Cleveland McEntire, Ph.D.
Research Scientist
Dept. of Science and Technology Projects
Washington, D.C.

Rosetta Newsome, Ph.D.
Director, Science and Communications
Chicago, Ill.

Fred Shank, Ph.D.
Vice President, Science, Communications, and Government Relations
Washington, D.C.

Introduction

The availability of antibiotics to treat infectious diseases has radically improved human and animal well being, and to a minor degree, plant health. Paradoxically, this very success threatens the future utility of antibiotics. The discovery of penicillin in 1940 ushered in the era of "modern medicine." Numerous antimicrobials, including most structural classes of antibiotics were discovered during 1920 to 1970. Chemical modification of many of these compounds led to new entities with superior activities. Because of the great success in antibiotic discovery, by the late 1970s, many proclaimed that the war on infectious diseases had been won, leading ultimately to de-emphasis of antibiotic discovery during the 1980s and a decline in the 1990s. At the same time, however, widespread antibiotic resistance was emerging and resulting in impaired treatment of human diseases (Neu 1992). As the genomes of bacteria, especially pathogens, have become increasingly available, the prospect of using them to identify new targets for antibiotic discovery has renewed interest in such investigations between the public sector and large pharmaceutical and biotechnology companies. Many of the larger companies and much of the public sector, however, have redirected research efforts toward noninfectious disease targets.

All uses of antibiotics in human medicine and animal husbandry create selective pressure that favors emergence of antibiotic resistance among microorganisms, which could undermine the effectiveness of the antibiotics and potentially give rise to a "postantibiotic" era. The selection for antibiotic-resistant bacteria in agricultural production environments and the subsequent impact on animal and human health has become a major concern and is the subject of many reports (Table 1). This document focuses on the use of antimicrobial agents to control bacteria in the food system; other microorganisms are considered as well, however. This document builds upon the IFT Scientific Status Summary "Resistance and Adaptation to Food Antimicrobials, Sanitizers, and Other Process Controls" (IFT 2002a), to inform readers about the various types of antimicrobial agents, including antibiotics, food antimicrobial agents, and sanitizers that are used at various stages of the food system, and the mechanisms that microorganisms, particularly foodborne pathogens, have for surviving the stress of exposure to these substances in their environments. Trends in antimicrobial resistance, and the resultant human health, economic, and clinically relevant environmental impacts are also addressed.

Classification of antimicrobials

For the purposes of this report, "antimicrobial" is a general term used broadly to refer to any compound, including antibiotics, food antimicrobial agents, sanitizers, disinfectants, and other substances, that acts against microorganisms. The definitions and use of each of these terms differ among various groups. Legal definitions exist for use in a regulatory context.

The term antibiotic is used in this report to refer to drugs used to treat infectious disease in humans, animals, or plants, by inhibiting the growth of or destroying microorganisms; such substances may be naturally occurring, semisynthetic, or synthetic. Antibiotics are also used in food animals to prevent infectious disease and improve the efficiency of feed utilization. Within the antibiotic classification are synthetic antimicrobials such as quinolones, that differ from other substances such as streptomycin, which are natural products or fermentation derived antibiotics. Antibiotics are legally classified as such only when used in humans. They are classified as "veterinary antimicrobial drugs" when used in animals and as "pesticides" when used in plants.

"Biocide" is a general term that refers to chemical agents, such as disinfectants and sanitizers, which are usually broad spectrum. Because biocides vary in antimicrobial activity, other terms may be used to more specifically describe the nature of the antimicrobial activity. For example, terms ending in the suffix "-static," such as "bacteriostatic," are used for agents that inhibit microbial growth without killing the microbes, and terms with the suffix "-cidal," such as "fungicidal," refer to agents that kill the target microbe (McDonnell and Russell 1999). "Disinfectants" destroy or irreversibly inactivate infectious fungi and vegetative bacteria (growing or nonsporeforming), and are used in hospitals, food processing facilities, restaurants, and elsewhere for general purposes (EPA 2005). In the legal connotation, disinfectants include "any oxidant, including but not limited to chlorine, chlorine dioxide, chloramines, and ozone, added to water in any part of the treatment or distribution process, that is intended to kill or inactivate pathogenic microorganisms" (40 CFR §141.2). "Sanitizers," comprised of 2 categories—no-rinse food contact surface sanitizers and nonfood contact surface sanitizers—refer to substances that reduce microbial contamination and destroy vegetative pathogens of public health significance on treated inanimate surfaces. "Sterilants," such as peroxyacetic acid, refer to substances that eliminate all forms of microbial life, including bacterial spores, fungi, and viruses. IFT uses the legal connotation "food antimicrobial agent" to refer to antimicrobial substances, such as nisin and other bacteriocins, including mold inhibitors, which are used to preserve food by preventing microbial growth and subsequent spoilage. Antibiotics cannot legally be used as food additives; thus, they are specifically excluded from this classification.

Classification of resistance

Discussion of antimicrobial resistance, by necessity, must include defining what is meant by resistance. While it would seem that defining resistance would be a simple matter, many definitions exist (Davison and others 2000). Resistance to most traditional, regulatory-approved, or naturally occurring food antimicrobial agents is difficult to characterize because of the lack of a precise definition for such resistance. From a functional perspective, resistance correlates with failure of a given antimicrobial treatment, whereas from a laboratory perspective, resistance is denoted through a "Minimal Inhibitory Concentration" (MIC)[2] value that exceeds a threshold value, which may or may not be associated with a clinical outcome. Chapman (1998) stated that a microorganism is resistant if it exhibits "significantly reduced susceptibility" when compared with that of the "original isolate" or a group of sensitive strains. In this report, resistance

[2] MIC is the lowest concentration of an antimicrobial drug, expressed in μg/ml or mg/L, which under defined in-vitro conditions within a defined period of time inhibits growth of the microbial inoculum.

Table 1—Reports of antimicrobial use, resistance, and human health impact

Date	Country or International	Report source	Report title	URL address (if applicable)
1969	United Kingdom	English Parliament	The Report to Parliament by the Joint Committee on Antibiotic Uses in Animal Husbandry and Veterinary Medicine ("Swann Report")	
1980	United States	National Research Council (NRC)	The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feed	http://fermat.nap.edu/catalog/21.html
1981	United States	Council for Agricultural Science & Technology	Antibiotics in Animal Feeds, Report 88	
1981	United States	Institute of Medicine (IOM)	Human Health Risks with the Subtherapeutic Use of Penicillin or Tetracyclines in Animal Feed	
1989	United States	IOM *Committee on Human Health Risk Assessment of Using Subtherapeutic Antibiotics in Animals*	Human Health Risks with the Subtherapeutic Use of Penicillin or Tetracyclines in Animal Feeds	
1997	International	World Health Organization (WHO)	The Medical Impact of the Use of Antimicrobials in Food Animals	http://whqlibdoc.who.int/hq/1997/WHO_EMC_ZOO_97.4.pdf
1998	United Kingdom	UK Ministry of Agriculture, Fisheries, and Food	A Review of Antimicrobial Resistance in the Food Chain	
1998	United States	United States Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM)	A proposed framework for evaluating and assuring the human safety of the microbial effects of antimicrobial new drugs intended for use in food-producing animals	http://www.fda.gov/cvm/VMAC/antimi18.html
1998	International	WHO	Use of Quinolones in Food Animals and Potential Impact on Human Health: Report and Proceedings of a WHO Meeting	http://www.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=93&codcch=157
1999	European Union	The European Agency for the Evaluation of Medicinal products	Antibiotic Resistance in the European Union Associated with Therapeutic Use of Veterinary Medicines	
1999	European Union	EU Scientific Steering Committee	Opinion of the Scientific Steering Committee on Antimicrobial Resistance	http://www.europa.eu.int/comm/dg24/health/sc/ssc/out50_en.html
1999	United States	FDA	Risk Assessment on the Human Health Impact of Fluoroquinolone-resistant Campylobacter Associated with Consumption of Chicken	http://www.fda.gov/cvm/Risk_asses.htm (revised as of January 5, 2001)
1999	United States	NRC *National Academy of Sciences Committee on Drug Use in Food Animals and the Panel on Animal Health, Food Safety, and Public Health*	The Use of Drugs in Food Animals: Benefits and Risks	http://fermat.nap.edu/catalog/5137.html
1999	United States	U.S. General Accounting Office (GAO)	Food Safety: The Agricultural Use of Antibiotics and Its Implications for Human Health	http://www.gao.gov/archive/1999/rc99074.pdf
1999	United Kingdom	Advisory Committee on the Microbiological Safety of Food	Report on Microbial Antibiotic Resistance in Relation to Food Safety	http://www.poultry-health.com/library/antimicrobials/acmsf996.htm (a synopsis)
1999	Australia	Joint Expert Advisory Committee on Antibiotic Resistance	The Use of Antibiotics in Food-Producing Animals: Antibiotic Resistant Bacteria in Animals and Humans	http://www.health.gov.au/internet/wcms/publishing.nsf/content/2A8435C711929352CA256F180057901E/$File/jetacar.pdf
1999	European Union	European Commission	Opinion of the Scientific Steering Committee on Antimicrobial Resistance, May 28, 1999	
1999	International	WHO	The Medical Impact of the Use of Antimicrobials in Food Animals	

(*continued on next page*)

Table 1—Continued

Date	Country or International	Report source	Report title	URL address (if applicable)
2000	United States	Centers for Disease Control and Prevention *Interagency Task Force on Antimicrobial Resistance*	A Public Action Health Plan to Combat Antimicrobial Resistance	http://www.cdc.gov/drugresistance/actionplan/
2000	International	WHO	WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food	http://www.who.int/salmsurv/links/en/GSSGlobalPrinciples2000.pdf
2000	International	Food and Agriculture Organization of the United Nations (FAO)/WHO *Codex Committee on Residues of Veterinary Drugs in Foods*	Antimicrobial Resistance and the Use of Antimicrobials in Animal Production	ftp://ftp.fao.org/codex/ccrvdf12/rv00_04e.pdf
2001	International	Office International Des Epizooties (OIE)	Antimicrobial Resistance: Reports prepared by the OIE Ad Hoc Group of Experts on Antimicrobial Resistance	http://www.oie.int/eng/publicat/ouvrages/a_106.htm
2001	International	WHO	WHO Global Strategy for Containment of Antimicrobial Resistance	http://www.who.int/drugresistance/WHO_Global_Strategy_English.pdf
2001	International	WHO	Monitoring Antimicrobial Usage in Food Animals for the Protection of Human Health	http://www.who.int/salmsurv/links/en/GSSMontitoringAMRuseOslo.pdf
2002	United States	Alliance for the Prudent Use of Antibiotics	The Need to Improve Antimicrobial Use in Agriculture: Ecological and Human Health Consequences ("FAAIR Report")	http://www.journals.uchicago.edu/CID/journal/contents/v34nS3.html
2002	Canada	Veterinary Drugs Directorate, Health Canada *Report of the Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health*	Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health	http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/pubs/amr-ram_final_report-rapport_06-27_e.pdf
2003	International	WHO *Department of Communicable Diseases, Prevention and Eradication and Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens*	Impacts of Antimicrobial Growth Promoter Termination in Denmark	http://www.who.int/salmsurv/en/Expertsreportgrowthpromoterdenmark.pdf
2004	International	FAO, OIE, and WHO	Joint FAO/OIE/WHO Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment	http://www.who.int/foodsafety/publications/micro/en/amr.pdf
2004	International	FAO, OIE, and WHO	Second Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Management Options	http://www.who.int/foodsafety/publications/micro/en/oslo_report.pdf
2004	United States	GAO	Federal Agencies Need to Better Focus Efforts to Address Risk to Humans from Antibiotic Use in Animals	http://www.gao.gov/highlights/d04490high.pdf

means "temporary or permanent ability of a microorganism and its progeny to remain viable and/or multiply under conditions that would destroy or inhibit other members of the strain" (Cloete 2003). These terms and the different types of resistance are described below.

As Courvalin (2005) describes, resistance can result from mutations in housekeeping structural or regulatory genes, or alternatively, horizontal acquisition of foreign genetic information. In some cases, resistance may manifest through multiple mechanisms. For example, three different strategies are thought to be involved in resistance to tetracycline (Schnappinger and Hillen 1996). Resistance can also be intrinsic, that is microorganisms without known exposure to antimicrobial agents may be resistant to some agents (see below).

If a resistant strain is isolated from an environment containing an antimicrobial or prepared in the laboratory by exposure to increasing concentrations of an antimicrobial, resistance may be due to a genetic alteration or a temporary adaptation. It may be that temporary adaptation to an antimicrobial through some type of homeostatic mechanism plays a much larger role than true genetic mutation among food-related antimicrobials. To date, research on resistance to food antimicrobials has focused

almost exclusively on innate or intrinsic mechanisms of the target microorganisms.

Innate (Intrinsic) resistance. As is the case for a natural property of a microorganism, innate resistance is chromosomally controlled (Russell 1991). Innate resistance is related to the general physiology or anatomy of a microorganism and stems from preexisting mechanisms or properties. This type of resistance is most likely responsible for differences in resistance observed among different types, genera, species, and strains of microorganisms in identical environmental conditions and concentrations. Innate resistance may stem from the complexity of the cell wall, efflux mechanisms (means by which microbes pump antimicrobials out of the cell [Gilbert and McBain 2003]), or enzymatic inactivation of the antimicrobial (Russell 2001). For example, because of the complexity of their cell walls, Gram-negative bacteria generally have a higher level of resistance to antibacterial agents than typical Gram-positive bacteria (Russell and Chopra 1996). More specifically, Gram-negative bacteria are innately resistant to penicillin G by virtue of their double membrane structure, which prevents the antibiotic from accessing the cell wall target. Similarly, *Mycobacterium* species are more resistant than other nonsporeforming bacteria due to high lipid content in their cell walls and comparatively high hydrophobicity. Other bacteria—*Bacillus*, *Pseudomonas*, *Corynebacterium*, *Micrococcus*, and the fungus *Aspergillus* have innate resistance to benzoate because they are capable of metabolizing the compound to succinic acid and acetyl coenzyme A (Chipley 1993). Innate resistance is not considered an important clinical problem because antibiotics were never intended for use against intrinsically resistant bacteria.

There are certain circumstances in which antimicrobial agents do not adversely affect bacteria that are generally susceptible to the particular agent. Because the efficacy of most food antimicrobials and sanitizers is dependent upon and influenced by the conditions of the application, some situations may permit bacterial resistance that would not have occurred otherwise (IFT 2002a). Exposure conditions, such as the environmental conditions (temperature, pH, and food composition) of the antimicrobial application, or interaction of the antimicrobial with components of the suspension medium or food product can influence the efficacy of the antimicrobial agent (Davidson 2001). For example, organic matter can quench the hypochlorite ion and therefore eliminate its efficacy at killing generally susceptible bacterial populations (Kotula and others 1997). However, microorganisms that are generally susceptible to antibiotics can themselves also become temporarily resistant to an antimicrobial through activation of silent, resident gene(s) that confer this resistance. A good example of this occurring is observed with the survivability of biofilm-associated cells versus planktonic (free floating/living) cells (Frank and Koffi 1990; Mosteller and Bishop 1993; Mustapha and Liewen 1989). Microbial cells in biofilms exhibit resistance primarily through the protection provided by extracellular materials such as exopolysaccharides. Also, nongrowing bacterial cells are resistant to many antibiotics that target cell wall synthesis. Once conditions again become favorable for growth, these bacterial cells become susceptible again to these cell wall inhibitors. Also, the few reports of resistance to food antimicrobial preservatives and sanitizers are attributed to microbial stress responses to sublethal stressors, such as low or high temperatures, acidity, osmolarity, low moisture, high atmospheric pressure, low oxygen or anaerobic conditions, gas atmospheres, competing bacteria, and low nutrient environments that trigger physiological changes and subsequently confer resistance to these compounds (Abee and Wouters 1999; Archer 1996; Samelis and Sofos 2003a; Sheridan and McDowell 1998; Sofos 2002a).

Acquired (Extrinsic) resistance. Acquired resistance results from genetic changes that occur through mutation of the antimicrobial's target site within the bacterium or acquisition of genetic material encoding resistance via plasmids[3] or transposons[4] containing integron sequences[5] (Roe and Pillai 2003; Russell 1991, 1996; Russell and Chopra 1996). Acquired resistance, the most common type of antibiotic resistance, has been well studied for antibiotics, but has not been well studied for food antimicrobial agents and sanitizers. Acquisition of genes for β-lactamase (an enzyme capable of breaking down and inactivating β-lactam antibiotics [penicillins and cephalosporins]) and mutation of one of the subunits of DNA gyrase (the target of fluoroquinolones) are examples of this type of resistance. Another example includes resistance of some microorganisms to sanitizing compounds, such as quaternary ammonium compounds (QACs), as a result of the presence of plasmid-encoded efflux pumps that remove the QACs (Russell 1997).

Although acquired resistance is of concern in the use of food antimicrobial agents and sanitizers, occurrence of such resistance appears to be rare. Unlike antibiotics, which generally have specific target sites, biocides (that is, disinfectants, sanitizers, antimicrobials) typically act nondiscriminately against multiple nonspecific targets (Bower and Daeschel 1999); thus, single mutations or biochemical alterations of cellular targets seldom confer resistance to biocides.

Adaptation. For certain types of antimicrobials, adaptation may be demonstrated by exposing a microorganism to a stepwise increase in concentration of the substance. This type of resistance, however, is often unstable; the microorganism may revert back to the sensitive phenotype when grown in an antimicrobial-free medium, termed "back-mutation" (Russell 1991). In the absence of selection pressure, the mutations associated with resistance may actually reduce fitness of the bacterial strain compared to the wild type, parental strain. Stabilizing, secondary, compensatory mutations are sometimes needed to maintain resistance and reduce fitness cost associated with the original "resistance" mutation.

Antimicrobial Applications

During food production and manufacturing, a variety of antimicrobials, including antibiotics, antifungals, sanitizers, and food preservatives, are applied to improve the efficiency of the system, and increase the safety and quality of the product. The multiple points throughout the ecosystem where antimicrobials may be used and subsequently impact the epidemiology of resistance are shown in Figures 1 and 2. Microorganisms encounter and are subjected to a variety of antimicrobial stressors as they move throughout the food system, from the environment to the plant, through food processing, shipping, distribution, storage, and into kitchen food preparation areas. The variety of antimicrobial uses at each of the various stages of the food system may create selective pressure that promotes resistance.

The major classes of antibiotics and their various uses in animals, plants, and humans are listed in Table 2. Detailed information on the mechanism of action of specific classes of antimicrobials can be found elsewhere (Prescott and others 2000; Walsh and others 2003). Some of the antibiotics and fungicides used in agriculture have identical chemical counterparts in human medicine. The majority of antibiotics used in food

[3] plasmid: self-replicating, extrachromosomal double-stranded, circular DNA, with exceptions
[4] transposon: genetic element that physically moves from one genetic position to another within the chromosome or plasmid in which it resides.
[5] integron sequences: genetic elements similar to transposons

animals belong to classes of antibiotics that are also used to treat human illness; these include tetracyclines, sulfonamides, penicillins, macrolides, fluoroquinolones, cephalosporins, aminoglycosides (gentamicin and kanamycin), chloramphenicols, and streptogramins (NARMS 2006).

Antibiotics are also used in companion animals, most often for treating dermatological conditions, ear infections, respiratory infections, urinary tract infections, and wounds. Applications in companion animals are addressed in Appendix 1.

Production agriculture

Animal husbandry. Foods of animal origin have been a mainstay of American agriculture. During the past half-century, food animal production has increased dramatically as a result of advances in science and technology, including the use of antibiotics in treating and preventing disease. Improvements in animal genetics, housing, nutrition, biosecurity, husbandry, and veterinary medicine, concurrent with more efficient business practices and economies of scale, have allowed food animal production to meet the demands of consumers. Antibiotics have been used in food animals (primarily cattle, swine, and poultry) for more than 50 years to treat, prevent, or control infectious disease, or to improve efficiency of feed utilization and weight gain (Gustafson and Bowen 1997). Specific information on antimicrobial agents used in animals can be found in the "Green Book" (listing the FDA-approved animal drug products) or the Feed Additive Compendium (Anonymous 2006a; FDA/CVM 1998). Administration of these veterinary drugs to food animals is a critical component of an overall management system that food animal producers use to secure the health and welfare of the animals and ensure the safety of the products that enter the food chain. Commensurate with

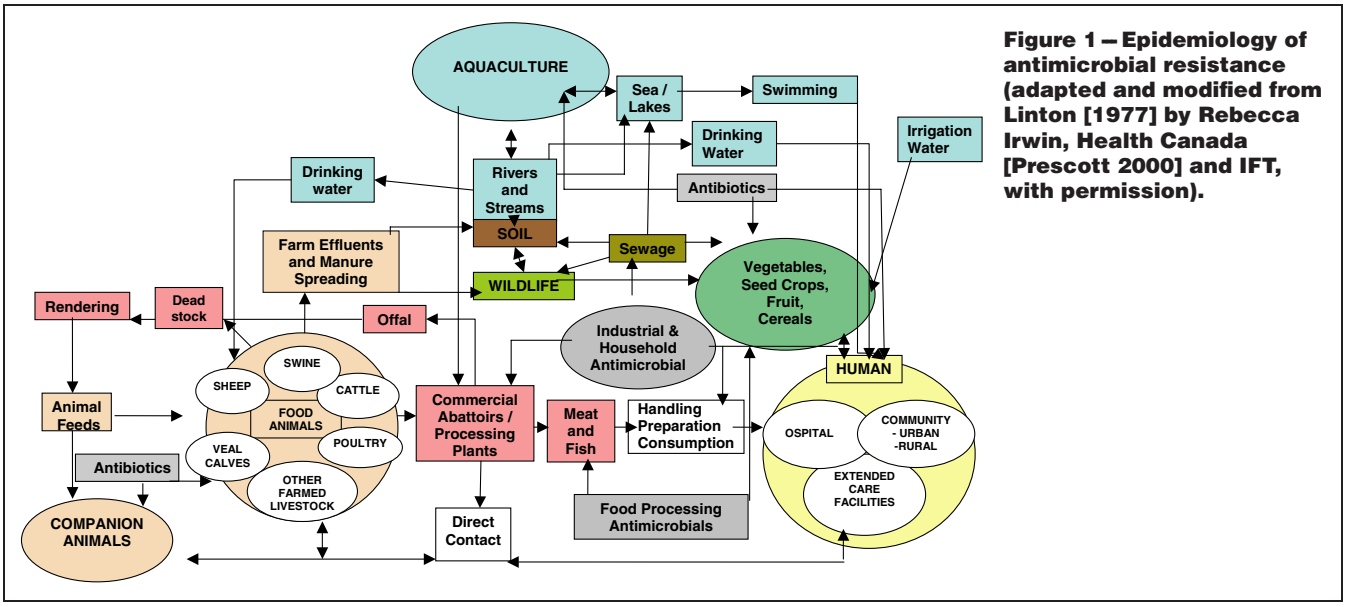

Figure 1—Epidemiology of antimicrobial resistance (adapted and modified from Linton [1977] by Rebecca Irwin, Health Canada [Prescott 2000] and IFT, with permission).

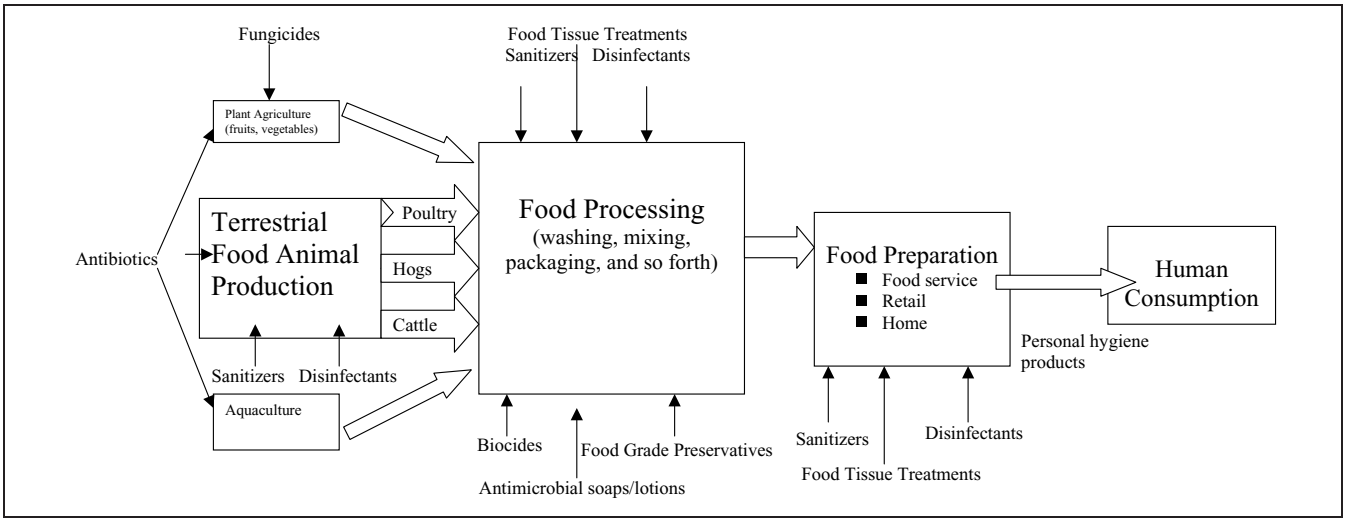

Figure 2—Application of antimicrobials from farm to table

Table 2—Examples of antimicrobial drugs and antibiotics, by major class, approved in the United States for animal, plant, or human use

Antimicrobial, drug class (selected examples)	Mode of action/ spectrum	Food animal use				Plant use	Human use
		Animal Species	Disease treatment	Disease prevention	Growth promotion		
Aminoglycosides (gentamycin, neomycin, streptomycin)	Inhibit protein synthesis/broad spectrum	Beef cattle, goats, poultry, sheep, swine	•	•		• (Certain plants)	•
Beta-lactams penicillins (amoxicillin, ampicillin)	Inhibit cell wall synthesis	Beef cattle, dairy cows, fowl, poultry sheep, swine	•	•	•		•
cephalosporins 1st generation (cefadroxil)	Broad spectrum						•
cephalosporins 2nd generation (cefuroxime)			•				
cephalosporins 3rd generation (ceftiofur)	Beef cattle, dairy cows, poultry, sheep swine		•	•	•		•
Chloramphenicol (Florfenicol)	Inhibit protein synthesis/ broad spectrum						•
	Inhibit protein synthesis/ broad spectrum	Beef cattle	•				
Cycloserines (cycloserine)	Inhibit cell wall synthesis/narrow spectrum						•
Glycopeptides (vancomycin)	Inhibit cell wall synthesis/narrow spectrum						•
Ionophores (monensin, salinomycin, semduramicin, lasalocid)	Disrupts osmotic balance /narrow spectrum	Beef cattle, fowl, goats, poultry, rabbits, sheep	•	•	•		
Lincosamides (lincomycin)	Inhibit protein synthesis/narrow spectrum	Poultry, swine	•	•			•
Macrolides (tylosin, tilmicosin erythromycin)	Inhibit protein synthesis/narrow spectrum	Beef cattle, poultry, swine	•	•	•		•
Monobactrams (aztreonam)	Inhibit cell wall synthesis broad spectrum						•
Polypeptides (bacitracin)	Inhibit cell wall synthesis narrow spectrum	Fowl, poultry, swine	•	•	•		•
				•	•		•
Fluoroquinolones (enrofloxacin, danofloxacin)	Inhibit DNA synthesis/broad spectrum	Beef cattle	•				•
Streptogramins (virginiamycin)	Inhibit protein synthesis/narrow spectrum	Beef cattle, poultry, swine	•	•	•	•	
Sulfonamides (sulfadimethoxine sulfamethazine sulfisoxazole)	Inhibit folic acid synthesis/broad spectrum	Beef cattle, dairy cows, fowl, poultry, swine, catfish, trout, salmon	•		•		•
Tetracyclines (chlortetracycline oxytetracycline tetracycline)	Inhibit protein synthesis/broad spectrum	Beef cattle, dairy cows, fowl, honey bees, poultry sheep, swine, catfish, trout, salmon, lobster	•	•	•	• (Certain plants)	•

(*Continued on next page*)

Table 2—Continued

Antimicrobial, drug class (selected examples)	Mode of action/ spectrum	Food animal use				Plant use	Human use
		Animal Species	Disease treatment	Disease prevention	Growth promotion		
Others							
Bambermycin	Inhibit cell wall synthesis/narrow spectrum	Beef cattle, poultry, swine		•	•		•
Carbadox	Inhibits DNA swine synthesis/narrow spectrum			•	•		
Novobiocin	Inhibits DNA gyrase/narrow spectrum	Fowl, poultry	•	•			•
Spectinomycin	inhibit protein synthesis/narrow	Poultry, swine		•			•

[a]Poultry includes at least one of the following birds: broiler chickens, laying hens, and turkeys.
[b]Fowl includes at least one of the following birds: ducks, pheasants, and quail.
(adapted and modified from GAO 1999)

increased food animal productivity is the inevitable shift to more intensive production systems, most notably in beef cattle, poultry and swine, to meet the expectations and needs of a growing number of people. Antibiotic use in food animals as an overall strategy to prevent and treat infectious disease is most relevant to antibiotic use in intensive production systems, in which the health of food animals is linked to consumer need for plentiful amounts of food animal products, food safety, and public health.

In modern production systems, food animals are generally raised in groups (NRC 1999). Typically chickens are raised in barns accommodating 10000 to 20000 birds, pigs are maintained in multiple-pen buildings, and beef cattle are raised outdoors in large pens in feed yards. Given the close proximity of the animals to one another (commingling), physiological and environmental stressors, and immature immune systems, any underlying viral infections, or bacterial respiratory or enteric diseases that may occur in a few animals can spread to others, including entire herds or flocks. Within the limits of the production system, and depending on the nature of the disease, the producer and/or veterinarian may intervene in such situations by medicating the entire group via the feed or water rather than treating each affected animal. Feed medication is more efficient for long-term prophylaxis, whereas medication of water is more effective for treating disease outbreaks due to its rapid intake and clinical response elicitation. Medicated water is also a more effective means for treating sick animals, which often continue to drink despite not continuing to eat. Administration of medication via water also allows large numbers of animals to be treated in an efficient manner, and avoids worker safety issues associated with injecting large numbers of animals.

Therapeutic uses. Therapeutic antimicrobial regimens include treatment, control, and prevention of disease (NCCLS 2002). Treatment is the administration of an antimicrobial to an animal or group of animals exhibiting frank, clinical disease (NCCLS 2002). Control is the administration of an antimicrobial to animals, usually as a herd or flock (metaphylaxis), in which morbidity and/or mortality has exceeded baseline norms, that is, early in the course of disease onset in the population. For example, as beef calves arrive at the feedlot, some of the animals disembarking from the truck may exhibit signs of clinical disease, for which treatment is necessary. While the other animals from the truck appear healthy, they have likely been exposed to the inciting pathogen and would otherwise "break" with disease if not also treated. The control concept is based on the premise that because the risk of disease spread from an individual animal or small group of diseased animals to the large susceptible population is substantial, it is appropriate that all animals be medicated. Prevention or prophylaxis is the administration of an antimicrobial to exposed at-risk healthy animals, generally in a herd or flock situation rather than on an individual animal basis, prior to the onset of a disease for which no etiologic agent has been cultured. An example of antimicrobial prophylaxis is the intramammary infusion of antibiotics to all dairy cows in a herd at the end of the lactation cycle, known as "dry-cow therapy," to prevent mastitis at parturition.

Occurrence of risk factors for a particular disease, herd/flock history, and the appearance of clinical signs in some animals may be sufficient indication that empirical antibiotic therapy is warranted to limit potential spread among an animal population. Empirical treatment is based upon the experience of the veterinarian or food animal producer, and involves consideration of such factors as animal species and its susceptibility to suspected pathogen(s), pathogen virulence, treatment cost, and any applicable antibiotic withdrawal times.[6] In such circumstances, a bacteriological diagnosis is most often made retrospectively from a necropsy specimen from a dead animal, although a culture from a live animal within the exposed population sometimes is recognized by analysis. Upon pathogen identification, the diagnostic laboratory will perform antimicrobial susceptibility testing, the results of which will further guide the veterinarian in antibiotic selection.

All of the newer injectable and water-soluble antibiotic products, including ceftiofur, enrofloxacin, and florfenicol, must be

[6] Withdrawal times are regulatory determinations of the Food and Drug Administration (FDA) concerning medication of all food-producing animals which sets forth the period of time in which an antibiotic cannot be administered to food animals prior to milk or egg collection or slaughter of the animal for human consumption. The regulations are designed to ensure that no unsafe concentration of drug residue is present in the food animals at the time of slaughter. Adherence to the drug withdrawal times can be ensured only through use as directed by the manufacturer on the drug label. Withdrawal times may vary dependent upon factors such as the species and age of the animals, as well as type of food commodity. Meat, meat products, milk, and eggs that are found to contain violative residues are condemned to ensure they do not enter the food chain.

obtained by prescription from or dispensed by a veterinarian. Antibiotic agents intended for growth promotion or therapeutic use in feed are usually incorporated into the feed at the feed mill and fed directly to the animals without direct veterinary involvement. An exception, however, is a prescription-like order signed by a veterinarian, through the Veterinary Feed Directive, that is processed and "filled" at the feed mill.

Extra-label[7] drug use is also a legal option for specific circumstances in food animal production. Such use of a drug differs from its approved labeling, which addresses species, indication, dosage levels, and frequency or route of administration. Under strict provisions that include a veterinarian-client-patient relationship, veterinary uses of extra-label drugs are acceptable to the FDA as long as the regulatory requirements are met, including that any tissue residues of the drug in meat or meat products are less than predetermined limits. Extra-label drug use, however, is not permitted for drugs added to feed (21 CFR §530).

Performance improvement uses. In the 1950s, it was shown that antibiotics administered at low levels for an extended time period promote growth rate and feed efficiency (growth promotion) in healthy livestock, primarily cattle, swine, and chickens (Jukes 1971). The beneficial effects of antibiotics on feed efficiency and growth rate have since been demonstrated for all major livestock species (Hays 1991). The use of an in-feed antibiotic for growth promotion occurs most often in young, growing animals. Use in older animals has a lessened effect. The use of antibiotics for growth promotion is intended to allow farmers to produce food animals at less cost because the amount of feed required for an animal to reach production weight is reduced.

A number of mechanisms for the growth promotion effects of antibiotics have been proposed. Possible mechanisms have been reviewed by Gaskins and others (2002) and Shryock (2000). The potential mechanisms are thought to be physiological, nutritional, and metabolic in nature and relate to antibiotic inhibition of the normal microflora, enabling more energy to be expanded for nutrient use and increased conversion to weight gain. Studies with germ-free animals have suggested that growth promotion results from antibacterial activities within the gastrointestinal system (Feighner and Dashkevicz 1987). Since the only known common factor among the various structurally and mechanistically distinct antibiotics used for growth promotion is the ability to kill bacteria, this mechanism seems plausible. Further, the three antibiotics (tetracycline, tylosin, and bacitracin) most commonly used for growth promotion act by inhibiting bacterial protein or cell wall synthesis. Moreover, the intestinal microflora of animals affects gut physiology in a number of ways, influencing for example, water uptake, immune response, and nutrient availability (Savage 1977).

Collier and others (2003) found that tylosin decreased total bacteria within the digestive tract and reported that the decrease may reduce host-related intestinal or immune responses, which would divert energy that could otherwise be used for growth. Modulation of the intestinal microflora of animals, resulting in selective enrichment for certain "optimal" bacteria, could enhance gut physiology by optimizing metabolism or nutrient uptake. Thus, it is also thought that the optimal microflora assist in maintaining animal health, and subsequently public health as well, by selectively excluding pathogens through either occupation of the physical intestinal microhabitat or acting as microbial antagonists. Collier and others (2003) also reported that the ability of tylosin to improve animal growth may relate to its apparent selection for lactobacilli, commensals[8] known to competitively exclude potentially pathogenic species from colonizing the intestine.

The use of antibiotics for growth promotion, however, has been a target for elimination. In the European Union (EU), growth promotion claims for human use class feed additive antibiotic labels were withdrawn in the 1990s, and nonhuman use class feed additive antibiotics followed in January 2006. In the United States some large restaurant corporations (for example, McDonalds, Oak Brook, Ill., U.S.A.) have developed antibiotic use policies that exclude human-use antibiotic classes for growth promotion purposes in flocks and herds of suppliers from whom they purchase poultry and beef products.

Poultry. The poultry industry is the most integrated of all of the major food animal industries in the United States. With integration, a single company controls the entire production cycle, from breeders to retail market. Approximately 8.4 billion chickens (broilers) and 264 million turkeys were produced in 2004 (USDA/NASS 2005). In most hatcheries, day-old chicks are injected with vaccines or an antibiotic, such as gentamicin or ceftiofur, to prevent opportunistic bacterial infections. Broiler chickens (typically six to eight weeks of age and five to eight pounds) are typically raised in pens containing 10000 to 20000 birds; turkeys are typically raised in groups of 5000 to 10000 (Lasley 1983; Lasley and others 1983). The majority of drugs used in poultry are administered via feed or water. Ionophores[9] or arsenicals are used as coccidiostats and antibiotics are used as growth promoters (NRC 1999).

Starter and grower rations may contain up to three drugs—a prophylactic coccidiostat, an antibiotic growth promoter, and an arsenical compound having both anticoccidiostat and growth-promoting properties. One or more drugs may be deleted from grower and finisher rations, however, to reduce cost and comply with drug withdrawal times to prevent tissue residues (NRC 1999). Table 2, which lists the antimicrobials approved for use in the United States, identifies a number of antibiotics (for example, bacitracin, bambermycin, chlortetracycline, penicillin, and virginiamycin) that are approved for use for growth promotion and feed efficiency in broilers, turkeys, and layers. Several antibiotics, administered as feed additives, are approved for treating intestinal infections, such as necrotic enteritis (caused by *Clostridium perfringens*) and coccidiosis (a common parasitic poultry disease caused by *Eimeria* species). Bacitracin and virginiamycin, for example, are used to treat necrotic enteritis, and monensin, salinomycin, narasin, and semduramicin are used to treat coccidiosis. Respiratory disease, such as air sacculitis caused by *Escherichia coli*, is treated with tetracycline. A variety of other antimicrobial agents are used for various conditions in poultry production (Merck 2003).

Swine. In 2004, 103 million hogs were slaughtered for food use (USDA 2005a). To control their environment and reduce disease, swine are often raised in confinement, sometimes from birth to slaughter (farrow to finish), or in age-segregated management systems where they are moved to different farms for various production stages (nursery, grower, and finishing, for example). Increasingly, management systems are undergoing transition to the all-in/all-out system in which pigs of similar ages are housed together to limit spread of infectious disease among animals with different age-dependent immune systems. Operations with more

[7] extra-label use: actual or intended drug use in an animal in a manner, such as increased dose or treatment duration, that is not in accordance with approved labeling, either because labeled drugs are unavailable for the condition or they are considered no longer effective

[8] Commensals include such bacteria as generic *Escherichia coli*, lactic acid bacteria, or *Enterococci* occurring naturally in the intestinal tracts of humans and animals or on raw foods or used as starter cultures for fermentation.

[9] ionophores: compounds that facilitate transmission of an ion, calcium for example, across a lipid membrane based on the definition from *Merriam Webster's Medline Plus*: www.nlm.nitgov/medlineplus

than 5000 head accounted for more than 75% of the swine in the United States in 2001, compared with only 27% in 1994 (USDA 2003). Several major pork production companies are fully integrated, but most production is still segmented.

The majority of drugs for swine are administered via feed or water. Breeding sows and pre-weaning pigs, however, are an exception, with antibiotics generally administered to individual animals. Most swine receive an antibiotic in feed ("starter rations") after weaning, when they are most vulnerable to infectious disease (caused by antecedent viral infections predisposing the animals to mycoplasma and/or bacterial superinfection) that may be related to the stress of weaning and movement within the production unit (Dewey and others 1997). Pneumonia is an important problem in swine production; antibiotics such as ceftiofur, tilmicosin, penicillin, lincomycin, tetracyclines, and tiamulin are used to treat and prevent clinical cases and outbreaks. Gentamicin, carbadox, tetracyclines and neomycin are sometimes used to control diarrhea caused by bacteria such as *E. coli* and *C. perfringens*. Ileitis (caused by *Lawsonia intracellularis*) may be treated with antibiotics such as lincomycin, tiamulin, or tylosin. Feed efficiency and growth promotion can be achieved with bacitracin, tylosin, virginiamycin, tetracyclines, and penicillin. A variety of other conditions, for which other antimicrobial agents are used, exist in swine production (Merck 2003).

Beef cattle. More than 37 million head of cattle were slaughtered in 2004 (USDA 2005a). In contrast to the highly integrated poultry industry, the beef cattle industry is still quite segmented, with many calves changing ownership and shipped multiple times during their lifetime. Calves from many sources are combined via auction or sale barns, transported, and commingled at the feed yard. Upon entering a feedlot, young cattle are given vaccinations against gastrointestinal and respiratory diseases, as well antihelminthic drugs. During stressful events, such as weaning or transportation and commingling, calves often develop pneumonia or diarrhea—major causes of mortality—and are often treated via individual or group medication.

In the U.S. beef industry, the majority of antibiotics are used on feedlots (USDA 2000). In 1999, the U.S. Dept. of Agriculture (USDA) conducted a survey of U.S. feedlots to determine antibiotic treatment practices. For treatment of individual animals, approximately 50% of feedlots used tilmicosin and/or florfenicol and/or tetracyclines as part of the initial therapy. The feedlots also used cephalosporins (38.1%), penicillins (31.1%), fluoroquinolones (32.1%), and macrolides (17.4%) for individual animal therapy. Approximately 41% of feedlots administered antibiotics for metaphylactic therapy; those most commonly used were tilmicosin, oxytetracyclines, and florfenicol (among 70.3%, 31.9%, and 22.1% of feedlots, respectively; USDA 2000). An estimated 83% of feedlots administered at least one antibiotic to cattle in feed or water for disease prophylaxis (tylosin for liver abscesses, for example) or to increase feed efficiency. A variety of other antimicrobial agents are used for a variety of conditions in beef cattle production (Merck 2003).

Dairy cattle and veal calves. There were 9.12 million cattle in dairy production in 2001 (USDA 2002). Dairy herd health is closely associated with milk production and economic sustainability. Therefore, maintenance of herd health is closely dependent upon disease prevention and therapeutic drug use for a range of diseases. Severe diarrhea and pneumonia are two main causes of morbidity and mortality in dairy heifers. Most dairy heifers are vaccinated against a range of gastrointestinal and respiratory diseases to minimize the need for antibiotics. Other conditions such as footrot and reproductive diseases may require antibiotic treatment specific to the diagnosis (Merck 2003). Administration of antibiotics to lactating cows, however, must be done with care to avoid milk residues. Mastitis is the most costly disease among dairy cattle, and intramammary infection is the most costly disease in U.S. food animal production (NRC 1999). Acute mastitis must be diagnosed in individual cows and can be treated with intramammary infusions of several antibiotics, for example, β-lactams, pirlimycin, and erythromycin. Except for mastitis caused by environmental pathogens (coliforms, for example), which does not always require antibiotic therapy, antibiotics to prevent mastitis are often administered through intramammary infusions at the beginning of the "dry (nonlactating) period" on a routine basis to all animals in the herd (Gibbons-Burgener and others 2000).

To reduce transmission of disease from the dam, the majority of dairy calves are separated from dams within 24 hours of birth and provided an initial feeding of colostrums, often pasteurized, from the initial milking to provide maternal antibodies and immunity. Most calves are housed in individual hutches or pens to control infection, and are fed milk or milk replacers (that may be medicated with an antibiotic) until weaning at 6 to 8 weeks of age, after which time they are generally housed in groups. The males and excess females are sometimes used for veal production.

The majority of veal calves are raised in the United States individually in stalls until they are 16 to 18 weeks of age. Due to their young age and confinement rearing, respiratory and gastrointestinal diseases are major causes of illness and death. Although a number of antibiotics are available for use, few data on the relative frequency of treatment with these antibiotics in the veal industry are available (Sargeant and others 1994). Milk-based liquid starter diets fed to veal calves usually contain antibiotics for disease prophylaxis, until about 4 to 6 weeks of age when they are fed a milk-based liquid grower diet that does not contain an antibiotic (NRC 1999).

Minor species (sheep, goats, and bison). In the United States, minor species are defined by exclusion, as animals other than cattle, horses, swine, chickens, turkeys, dogs, and cats. In January 2005 the U.S. inventory of sheep and lamb totaled 6.14 million head (2.84 million slaughtered for food use), compared with cattle and calf inventory in July 2004 of 103.6 million (USDA 2005a, 2005b).

Six antibacterial drugs are approved for use in sheep, one of which—chlortetracycline—is approved for growth promotion and feed efficiency (NRC 1999). The focus of antibiotic treatment in sheep is the prevention and control of respiratory diseases, including shipping fever. Methods for administering drugs to sheep flocks include incorporation into feed or water, injection, and oral dosing. Treatment methods in goats are similar to those in sheep except that goats tolerate oral drenching less well, and in the United States it is common for goats to be treated as individuals rather than as herds. As ruminants, these species also receive protocols for the prevention and treatment of mastitis. Two antibiotics, neomycin and penicillin/streptomycin, are approved for use for enteritis and various infections, respectively; four drugs are approved for use for coccidiosis and parasites (NRC 1999).

Currently there are approximately 350000 head of bison in North America (NBA 2005); about 30000 head were slaughtered for food production in the United States in 2004 (USDA 2005a). Use of antibiotics in bison production is generally discouraged; and occurs only for treatment purposes. The Source Verification Program of the National Bison Association, which provides the standards for "certified buffalo products," prohibits administration of low doses of antibiotics over a long period of time. Medicated feeds are only permitted at "treatment" levels prescribed by a veterinarian.

While there are several other minor species used for food production, because their contribution to antimicrobial resistance is relatively small they are outside the scope of this report.

Food Animal Slaughter

Food animal slaughtering facilities in the United States apply carcass sanitization or physical or chemical decontamination treatments immediately before and after hide removal, at the end of the dressing process (before carcass chilling), and potentially after chilling; EU regulations, however, do not allow use of chemical decontamination agents in slaughter facilities (Koutsoumanis and others 2006; Sofos 2002b; Stopforth and Sofos 2006).

Some chemical agents may be incorporated into cleaning or washing solutions to reduce hide contamination. Solutions evaluated for this purpose include cetylpyridinium chloride, lactic acid, sodium hydroxide, ethanol, trisodium phosphate, acidified chlorine, and phosphoric acid. Chemical dehairing is a patented cattle hide decontamination process that involves use of a sodium sulfide solution followed by neutralization with hydrogen peroxide. This process is expected to minimize the importance of animal hides as sources of environmental and carcass contamination (Sofos and Smith 1998; Stopforth and Sofos 2006).

A number of interventions exist for sanitizing or decontaminating carcasses or fresh meat and poultry in the United States. These include water or steam (that is, hot water, pressurized steam, steam-vacuum) and chemical solutions, especially organic acids. These interventions significantly reduce bacterial populations, including those of enteric pathogens such as *Escherichia coli* O157:H7 and *Salmonella*. Such bacterial reductions allow the industry to meet regulatory (USDA/FSIS 1996) and contractual criteria. Spraying or rinsing of carcasses with an organic acid solution (for example, lactic and acetic acids) before evisceration and chilling reduces total bacterial populations and pathogen prevalence, and may also result in residual antimicrobial activity during product storage (Koutsoumanis and Sofos 2004a; Koutsoumanis and others 2004; Sofos and Smith 1998). Although this intervention reduces the prevalence and probably the concentration of *E. coli* O157:H7 on meat carcasses, concern has been raised that the treatment may select for, lead to adaptation of, or enhance the inherent tolerance of pathogen cells to acid (Samelis and Sofos 2003a, 2003b). In vitro studies have indicated the potential for sublethal organic acid rinsing treatments, which depend upon pH, acid type, and exposure duration, to cause acid stressing and selection of acid-resistant survivors in fresh meat decontamination runoff fluids. A potential concern is that any survivors may create niches in the plant environment for cross-contaminating subsequent batches of fresh meat (Samelis and others 2001a, 2002a, 2002b, 2003, 2004a, 2005b).

Additional chemical solutions for fresh meat and poultry decontamination include chlorine-based compounds and trisodium phosphate, which are used in the poultry industry, and acidified (usually with citric or lactic acid) sodium chlorite, hydrogen peroxide, ozonated water, activated lactoferrin, and peroxyacetic acid-based preparations. A variety of other tested chemical compounds such as polyphosphates, benzoates, propionates, sodium hydroxide, sodium metasilicate, and sodium bisulfate have shown various rates of success for decontaminating meat and poultry (Sofos 2002b; Stopforth and Sofos 2006).

Within a multiple hurdle approach to microbial control, fresh meat decontamination may involve the simultaneous sequential application of treatments that act synergistically or additively. Described by Leistner and Gould (2002) and Sofos and Smith (1998), a hurdle technology approach is the application to food of multiple physical, chemical, and biological antimicrobial factors at individually sublethal levels, rather than as a single hurdle at a higher, lethal level. When used in proper combinations, sublethal levels of antimicrobials are adequate for pathogen control, that is, microbial inactivation or growth inhibition. The multiple hurdles are designed to collectively lead to pathogen inactivation through metabolic exhaustion or growth inhibition for a certain period of time (Leistner and Gould 2002). For example, in fresh meat decontamination, the multiple hurdle approach may involve the simultaneous (for example, warm acid solutions) or the sequential (for example, hide cleaning, carcass steam vacuuming, pre-evisceration carcass washing, hot water, steam treatment, and organic acid rinsing treatments before carcass chilling, spray chilling of carcasses, and post-chilling-before-boning chemical treatments) application of treatments (Stopforth and Sofos 2006).

Effectiveness of hurdles may depend on the number and type of treatments, their intensity, and application sequence. For example, lactic acid rinsing of beef after hot water washing is more effective for microbial reduction and, especially, control of microbial growth during storage than before hot water washing (Koutsoumanis and Sofos 2004a; Koutsoumanis and others 2004; Koutsoumanis and others 2006; Sofos and Smith 1998). Synergism of an acid-heat-dehydration hurdle system was shown effective for inactivating *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* inoculated pre- or post-drying on beef subsequently used to produce jerky, a North American dried meat snack (Calicioglu and others 2002a, 2002b, 2003a, 2003b, 2003c, 2003d; Yoon and others 2005). Selection of hurdles, their intensity, and sequence of application should aim at maximizing control without pathogen stress-adaptation or selection of resistant cells (Samelis and Sofos 2003a).

Aquaculture. Various types of aquaculture involving many different food-fish species are practiced worldwide. The extensive type of aquaculture practiced before 1980 has given way to more intensive pond, cage, net-pen, raceway (flow-through), and closed recirculating system culture. In the year 2000, salmon, tilapia and hybrid striped bass production in the United States reached 49 million, 20 million, and 10 million pounds, respectively (Carlberg and others 2000; Posadas 2003a, 2003b). Total production of channel catfish reached 630 million pounds and the rainbow trout industry produced approximately 46 million pounds of trout 12 inches or larger in 2003 (NWAC 2003; USDA 2004a).

Increase in demand and production capability has led to an increased concern about diseases, especially bacterial diseases. Antibiotics are only approved to treat disease as labeled 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 disease. Management and control of bacterial diseases are accomplished by administering medicated feeds or vaccines, and implementing improved husbandry practices. In the United States, only four antibiotics, Romet® (sulfadimethoxine/ormetoprim 5:1, Hoffman LaRoche, Nutley, N.J., U.S.A.), Terramycin® (oxytetracycline, Pfizer, Inc., U.S. Animal Health Operations, New York, N.Y.,

U.S.A.), sulfamerizine (no longer manufactured or available for aquaculture use), and Aquaflor® (florfenicol, Shering Plough Animal Health, Kenilworth, N.J., U.S.A.) are approved for use in aquaculture. Antimicrobials used in the U.S. aquaculture industry are regulated by the FDA.

In the United States, production of channel catfish, *Ictalurus punctatus,* is the largest and most economically important form of intensive aquaculture. A typical catfish farm contains broodfish holding and spawning ponds, a hatcher, fingerling nursery, and grow out ponds. The ponds are earthen-bottomed and typically 10 to 20 acres in size. Catfish production has increased from 500 fish/acre in the industry's infancy to current levels of 10000 fish/acre. Of the losses caused by infectious disease in food-size channel catfish, approximately 60% are the result of single or mixed infections of *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish (ESC), and *Flavobacterium columnare*, the causative agent of columnaris disease (Khoo 2001). Most catfish farmers are familiar with the clinical signs of the common bacterial diseases of catfish, and at the first sign of disease, a sample of sick fish is collected and shipped to the nearest aquatic diagnostic laboratory. Diagnostic laboratories typically culture the causative agents of disease and perform antibiotic susceptibility testing on bacterial pathogens.

Antibiotic use in catfish culture escalated in 1981 with the emergence of ESC, until approximately 1997, when management trends began to change. Sulfadimethoxine/ormetoprim 5:1 has traditionally been the most popular drug premix, because it is incorporated into a floating feed, but this situation may change with the approval in 2005 of florfenicol medicated feed. Oxytetracycline is only available in a sinking feed, which is less desirable because feeding activity is difficult to monitor (MacMillan 2003).

The practice of stocking and growing tilapia and hybrid striped bass at very high densities in closed recirculating aquaculture systems has led to the emergence of several bacterial pathogens, most notably *Streptococcus iniae,* as a limiting factor in production. Cumulative mortality rates in young fish can reach 75% in a matter of weeks although mortality is usually not as explosive as for other bacterial diseases of fish (Plumb 1999). Currently no antibiotics are approved by FDA for treating bacterial diseases in tilapia or hybrid striped bass.

The rainbow trout industry has greater maturity than many other forms of aquaculture and benefits from years of research on the diseases of salmonids and best management practices for those diseases. Many large trout producers have their own staff of fish pathologists who are responsible for maintaining the health of the fish stocks. The most prevalent bacterial diseases of rainbow trout are enteric redmouth disease (ERM) caused by *Yersinia ruckeri,* bacterial kidney disease caused by *Renibacterium salmoninarum*, furunculosis caused by *Aeromonas salmonicida* and coldwater disease caused by *Flavobacterium psychrophilum.* Asymptomatic carriers are common with ERM resulting in efficient disease spread. Once considered a major problem in the farm-raised trout industry, ERM is largely controlled today by good management practices and vaccination, although oxytetracycline medicated feeds have also been successfully used. Oxytetracycline medicated feeds have been used successfully at 50–75 mg/kg of fish/day for 10 days followed by a 21-day withdrawal period (Plumb 1999). ERM was one of the first fish diseases to be managed by vaccination. Current practices involve vaccination of 4 to 4.5 g fingerlings by immersion in a killed bacterin (suspension of killed or attenuated bacteria for use as a vaccine), which provides protection for 12 mo. The success of the ERM vaccine has resulted in greatly reduced mortality, reduced antibiotic usage, and reduced feed conversion rates in U.S. rainbow trout (Plumb 1999). Bacterial kidney disease remains difficult to manage, and is currently treated by chemoprophylaxis by injecting brood stock with 20 mg/kg erythromycin. Management of furunculosis involves the use of disease resistant strains of fish, destruction of infected fish, facility sanitation, and restrictions on use of eggs from infected broodstock. Sulfadimethoxine/ormetoprim medicated feeds have been used successfully at 50 mg/kg of fish/day for 5 d with a 42-d withdrawal period. Vaccines have not been as successful commercially because injectable vaccines are required to elicit adequate protection.

In salmonid mariculture (cultivation of marine organisms in their natural environment), vibriosis has been implicated as an important disease. Several species of vibrio bacteria, particularly *Vibrio anguillarum, V. ordalii,* and *V. salmonicida*, are responsible for the disease. Oxytetracycline has been used to treat vibriosis with variable results. Bivalent vaccines with antigenic components from *V. anguillarum* and *V. ordalii* are currently used with great success.

In the early 1990s, several mariculture ventures were established in brackish-water areas of south Louisiana where hybrid striped bass, *Morone saxatilis x M. chrysops,* and red drum, *Sciaenops ocellatus,* were cultured in cages, net-pens, and ponds. The emergence of *Photobacterium damselae subsp. piscicida* as an important marine bacterial pathogen of hybrid striped bass, led to the use of antibiotic medicated feeds in an attempt to control mortality (Hawke and others 2003). Oxytetracycline, sulfadimethoxine/ormetoprim 5:1, and amoxicillin at 50 mg/kg fish/day were used to treat outbreaks of *P. damselae* subsp. *piscicida* in red drum and in hybrid striped bass on mariculture farms. The antibiotics were used after filing for permission from the FDA but were unsatisfactory for several reasons—poor efficacy due to rapid onset of disease and anorexia of sick fish, recurrent infections following the use of antibiotics, and rapid development of antibiotic resistant strains of *P. damselae* subsp. *piscicida* due to acquisition of R-plasmids (Hawke and others 2003).

In many instances, medicated feeds have not proven to be efficacious in aquaculture for a variety of reasons. Individual fish infected with bacterial diseases tend to go off feed early in an epizootic and will not receive a therapeutic amount of the antibiotic. For antibiotic feeds to effectively control an outbreak of disease, the majority of fish in the population must be actively feeding for individuals to receive a therapeutic dose. For this reason, early diagnosis and initiation of therapy are paramount. Additionally, maintenance of good water quality and parasite control are important to keep feeding responses high.

Plant agriculture. The types of antimicrobials used in plant agriculture include antibiotics for control of certain bacterial diseases, and fungicides for control of fungi. Fungi and viruses are the most prevalent microorganisms causing diseases of plants; bacteria are relatively minor in importance, with some notable exceptions. Fruit trees account for most antibiotic use on plants in the United States (McManus 2000). In the United States, streptomycin and oxytetracycline have been used for more than 40 y as preventative treatments to control bacteria, primarily, affecting fruits and vegetables. Trees are generally sprayed during blossom time, when they are most susceptible to infection by *Erwinia amylovora* (causal agent of fire blight) and *Pseudomonas syringae* pathovar *papulans* (causal agent of apple blister spot). The edible fruit is not sprayed. Although streptomycin is registered by the EPA for use on 12 fruit, vegetable, and ornamental fruit crops, and oxytetracycline is registered for use on 4 fruit crops (Vidaver 2002), a limited number of fruit tree species—apple, pear, and peach—are treated in such a manner by antibiotics.

Most antimicrobials used in plant agriculture are fungicides. The top 12 economically severe fungal diseases are: cereal rusts, cereal smuts, ergot of rye and wheat, late blight of potato, brown spot of rice, southern corn leaf blight, powdery and downy mildews of grapes, downy mildew of tobacco, chestnut blight,

Table 3—Sanitizers commonly used in the food industry (Davidson and others 2005; McDonnell and Russell 1999)

Active ingredient	Environmental surfaces	Food contact surfaces	Food tissues	Restroom	Handcare
Alcohols[a]	+	+			+
Oxidizing compounds[b]	+	+			
Hypochlorite	+	+	+	−	−
Quaternary ammonium compounds	+	+	±	+	−
Phenolics	−	−	−	+	−
Acid anionics	+	+	−	−	−
Acidified sodium chlorite	±	±	+	−	−
Chlorine dioxide	+	+	+	−	−
Triclosan	−	−	−	−	+
Para-chloro-meta-xylenol	−	−	−	−	+
Chlorhexidine	−	−	−	−	+

[a]Includes ethyl alcohol (ethanol, alcohol), isopropyl alcohol (isopropanol, propan-2-ol), and n-propanol.
[b]Includes hydrogen peroxide and peracetic acid.

Dutch elm disease, and pine stem rusts. Some of these diseases are worldwide and some are more restricted, due to host and climate (Agrios 2005).

Of the approximately 135 fungicides in 40 chemical classes (FRAC 2003), a large number are chemically classified as azoles. These popular fungicides are relatively cheap, have broad spectrum systemic activity for both preventative and curative effects, and are relatively stable (Hof 2001). The azoles are effective against mildews and rusts of grains, fruits, vegetables, and ornamentals; powdery mildew in cereals, berry fruits, vines and tomatoes; leaf spots and flower blights in flowers, shrubs and trees, and several other plant pathogenic fungi (Hof 2001). At present, there are no cross-over chemicals with those used in human medicine to treat serious 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 14α-demethylase) is the same (Dismukes 2000). Fungicide resistance in plant pathogens may be of concern to those treating medical mycoses.

Residues of antibiotics and fungicides on fruits and vegetables are monitored by the Environmental Protection Agency (EPA); the residues have not been considered of concern with respect to antimicrobial resistance. Treated microorganisms, however, may be present on fruit and produce. Thus, antimicrobial resistance of plant pathogens and resistance of microbes in the treated environment raise questions about the potential for compromise in the use of these antimicrobials in human disease treatment.

Genes coding for antibiotic resistance have been used as markers in transgenic plant production, which is used to indirectly recover the desired trait(s), that is trait(s) not previously achievable through conventional plant breeding. Thus, a desired trait from an unrelated plant, animal, or microbial source may be added to a plant's replication machinery in single-cell technology, but the transformed cells may not be selectable directly when grown as tissue culture in vitro. After initial indirect selection, some markers can be eliminated as the plant is allowed to grow normally. These recombinant DNA derived plants have raised questions about the potential transfer of antibiotic resistance to animals or humans, although there has been no conclusive evidence of gene transfer from plant chromosomes to animals or humans. The risk of transfer of antibiotic resistance markers and the corresponding hazard was reviewed by Bennett and others (2004), and found to be "remote" and "slight." Nevertheless, under the impetus of the EU, genes expressing resistance to antibiotics used in medical or veterinary treatment as markers will be phased out between 2004 and 2008.

Food processing

Several different types of antimicrobial agents (Tables 3 and 4) are used in food manufacturing to either clean or sanitize to prevent cross-contamination in food processing facilities, or ensure food quality and safety. Food antimicrobials were traditionally used to prevent food spoilage, and only recently have been applied to control pathogen growth. Unlike the approval process for use of antibiotics in animals, which requires a risk assessment of resistance acquisition, the potential for the development of resistance to food antimicrobial agents is not considered during their approval for food use.

Cleaning and sanitation. Equipment surfaces and the surrounding environment inevitably become soiled and require cleaning during food processing. In addition to detergents and soaps, antibacterial agents (biocides) are used as sanitizers, disinfectants, and handcare products throughout the food system. These substances are used to reduce the level of microorganisms on food contact surfaces, in food formulations, on ready-to-eat (RTE) food product surfaces, environmental surfaces, food tissue surfaces, and human skin. Formulations for these uses contain one or more antibacterial agents, commonly referred to as active ingredients, as well as other components including surfactants, pH buffering agents, and water conditioning agents. The active ingredients of sanitizers and various common uses in the food industry are shown in Table 3. Overviews of the cellular targets and inactivation mechanisms of biocides are provided in Figure 3 and 4.

Detergents may be classified into inorganic alkali (sodium hydroxide and sodium carbonate, for example), inorganic and organic acids (phosphoric and citric acids, for example), surface-active agents (for example, synthetic detergents—either anionic, cationic, non-ionic, or amphoteric [capable of reacting chemically as either an acid or base]), and sequestering agents (polyphosphates, ethylenediamine tetra acetic acid [EDTA, for example]). Modern detergents contain a mixture of different chemicals, each contributing to the desired properties of the formulation. Sanitizers used in the food industry can be classified into chlorine releasing compounds, QACs, iodophors[10] and amphoteric compounds.

Quality and safety. Sanitization or decontamination treatments, similar to those applied on raw beef, may also be used for fresh produce (Beuchat and Ryu 1997; Taormina and others 1999).

[10] iodophor: complex of iodine and a surface-active agent that releases iodine gradually and serves as a disinfectant

Table 4—FDA-approved food antimicrobials (IFT 2002a)

Compound(s)	Microbial target	Primary food applications	Title 21 CFR designation[a]
Acetic acid, acetates, diacetates, dehydroacetic acid	Yeasts, bacteria	Baked goods, condiments, confections, dairy products, fats/oils, meats, sauces	184.1005, 182.6197, 184.1754, 184.1185, 184.1721, 172.130
Benzoic acid, benzoates	Yeasts, molds	Beverages, fruit products, margarine	184.1021, 184.1733
Dimethyl dicarbonate	Yeasts	Beverages	172.133
Lactic acid, lactates	Bacteria	Meats, fermented foods	184.1061, 184.1207, 184,1639, 184.1768
Lactoferrin	Bacteria	Meats	[b]
Lysozyme	*Clostridium botulinum*, other bacteria	Cheese, casings for frankfurters, cooked meat and poultry products	184.1550[c]
Natamycin	Molds	Cheese	172.155
Nisin	*Clostridium botulinum*, other bacteria	Cheese, casings for frankfurters, cooked meat and poultry products	184.1538[d]
Nitrite, nitrate	*Clostridium botulinum*	Cured meats	172.160, 172.170, 172.175, 172.177
Parabens (alkyl esters (propyl, methyl, heptyl) of *p*-hydroxybenzoic acid)	Yeasts, molds, Gram-positive bacteria	Beverages, baked goods, syrups, dry sausage	184.1490, 184.1670, 172.145
Propionic acid, propionates	Molds	Bakery products, dairy products	184.1081, 184.1221, 184.1784
Sorbic acid, sorbates	Yeasts, molds, bacteria	Most foods, beverages, wines	182.3089, 182.3225, 182.3640, 182.3795
Sulfites	Yeasts, molds	Fruits, fruit products, potato products, wines	Various

[a]Food and Drug Administration designations in Title 21 of the *Code of Federal Regulations*. Food antimicrobials approved by the U.S. Department of Agriculture's Food Safety and Inspection Service for use in meat products are listed in sections 424.21 and 424.22 of Title 9 of the CFR.
[b]FDA/CFSAN GRAS notice 000067, Oct. 2001.
[c]FDA/CFSAN GRAS notice 000064, Apr. 2001.
[d]FDA/CFSAN GRAS notice 000065, Apr. 2001.

Combinations of thermal (hot water or steam) and chemical interventions (organic acid solutions) in the form of sprays or rinses are used successfully as sanitizing or decontaminating treatments on fresh produce to reduce overall microbial contamination and prevalence of pathogenic bacteria (Sofos 2002b; Sofos and Smith 1998; Stopforth and Sofos 2006).

Processing and preservation technologies involving manipulation of physical, chemical, and biological factors are used, often in combination, by food processors. The objective of their use is to ensure the stability and safety of foods by inactivating or inhibiting growth of spoilage and pathogenic microorganisms. For example, various combinations and sequences of sublethal hurdles in RTE meat and poultry products may also be applied to control post-lethality processing contamination with *L. monocytogenes* during product storage (see side bar), as required by new U.S. Dept. of Agriculture Food Safety and Inspection Service regulations (USDA/FSIS 2003).

Chemical preservatives and treatments. While some chemical food preservatives, such as common table salt, nitrites, and sulfites, have been in use for hundreds of years, most others have been extensively applied only in recent decades. Food preservatives used to prevent food deterioration caused by microbial growth are termed "food antimicrobial agents." The historical function of food antimicrobial agents is inhibition of spoilage microorganisms and extension of shelf life. The use of food antimicrobial agents to control pathogens is more recent and is increasing (Davidson and Zivanovic 2003). Food antimicrobial agents are generally not used alone to control foodborne pathogens, but are included as components of the multiple hurdle approach to microbial control. Exposure of *E. coli* O157:H7, *Salmonella,* or *L. monocytogenes*–inoculated apple slices or other produce to ascorbic and citric acid solutions, for example, enhanced destruction of the pathogens during subsequent drying (Burnham and others 2001; Derrickson-Tharrington and others 2005; DiPersio and others 2003, 2004; Yoon and others 2004). Other common applications of food antimicrobials include use of sodium nitrite to inhibit *Clostridium botulinum* in cured meats if product temperature abuse occurs, organic acid solutions as spray sanitizers to control pathogens on beef carcasses, nisin and lysozyme to control *C. botulinum* in pasteurized process cheese, and lactate and diacetate for *L. monocytogenes* control in processed RTE meat and poultry products (USDA/FSIS 2000).

Naturally occurring antimicrobials. Food antimicrobial agents may be classified as traditional or naturally occurring (Davidson 2001). Traditional food antimicrobial agents, listed in Table 4, undergo review and approval for food use by many international regulatory agencies. Naturally occurring antimicrobials, however, which include compounds from microbial, plant, and animal sources (Table 5) are limited in approval and applications (Sofos and others 1998). Nisin, natamycin, lactoferrin, and lysozyme are among the few naturally occurring substances that are approved by regulatory agencies in some countries for direct application to foods.

Food biopreservation uses natural or controlled microflora and/or their antibacterial metabolic end products to interfere with undesirable microorganisms. Lactic acid bacteria (LAB), for example, occur either in the initial natural microflora of fermented or other foods or are added as starter cultures, where their growth dominates over that of other microbes during fermentation or retail case display and home refrigeration (vacuum-packaged meat, for example). Growth of LAB interferes with spoilage and pathogenic bacteria through nutrient and oxygen depletion, and production of inhibitory metabolic substances such as lactic and acetic acid, acetoin, diacetyl, hydrogen peroxide, reuterin, and bacteriocins (Koutsoumanis and Sofos 2004a; Koutsoumanis and others 2006).

Controlling *L. monocytogenes* in Ready-to-Eat (RTE) Foods

Recalls of RTE meat and poultry products and foodborne illness outbreaks involving fatalities attributed to *L. monocytogenes* led to the establishment of a new regulation for controlling the pathogen in meat and poultry products that may become contaminated after processing, during slicing and packaging, and in which their growth may be supported during product distribution and storage (USDA/FSIS 2003). According to the regulation, manufacturers of sensitive RTE meat and poultry products should select one of three alternative approaches for preventing contamination and inactivating or controlling the pathogen's growth during storage. In addition to physical processes (for example, heat, high hydrostatic pressure), the alternatives may be based on chemical compounds applied as antimicrobial agents or sanitizers. Substances such as potassium or sodium lactate, sodium acetate, sodium or potassium diacetate, nisin, acetic acid, lactic acid, sodium or potassium benzoate or sorbate, acidic calcium sulfate, and buffered citrate applied as formulation ingredients or postprocessing solutions are effective against the pathogen in such RTE meat and poultry products. The most common approach for controlling *L. monocytogenes* in RTE meat and poultry products combines sodium or potassium lactate with sodium diacetate in the product formulation (Tompkin 2002). Alternative antimicrobial approaches may be based on combinations of physical and chemical antimicrobial hurdles applied as formulation ingredients during processing, or as postlethality treatments, including spraying or dipping solutions during packaging (Barmpalia and others 2004, 2005; Bedie and others 2001; Geornaras and others 2005; Samelis and others 2001c, 2002c, 2005a).

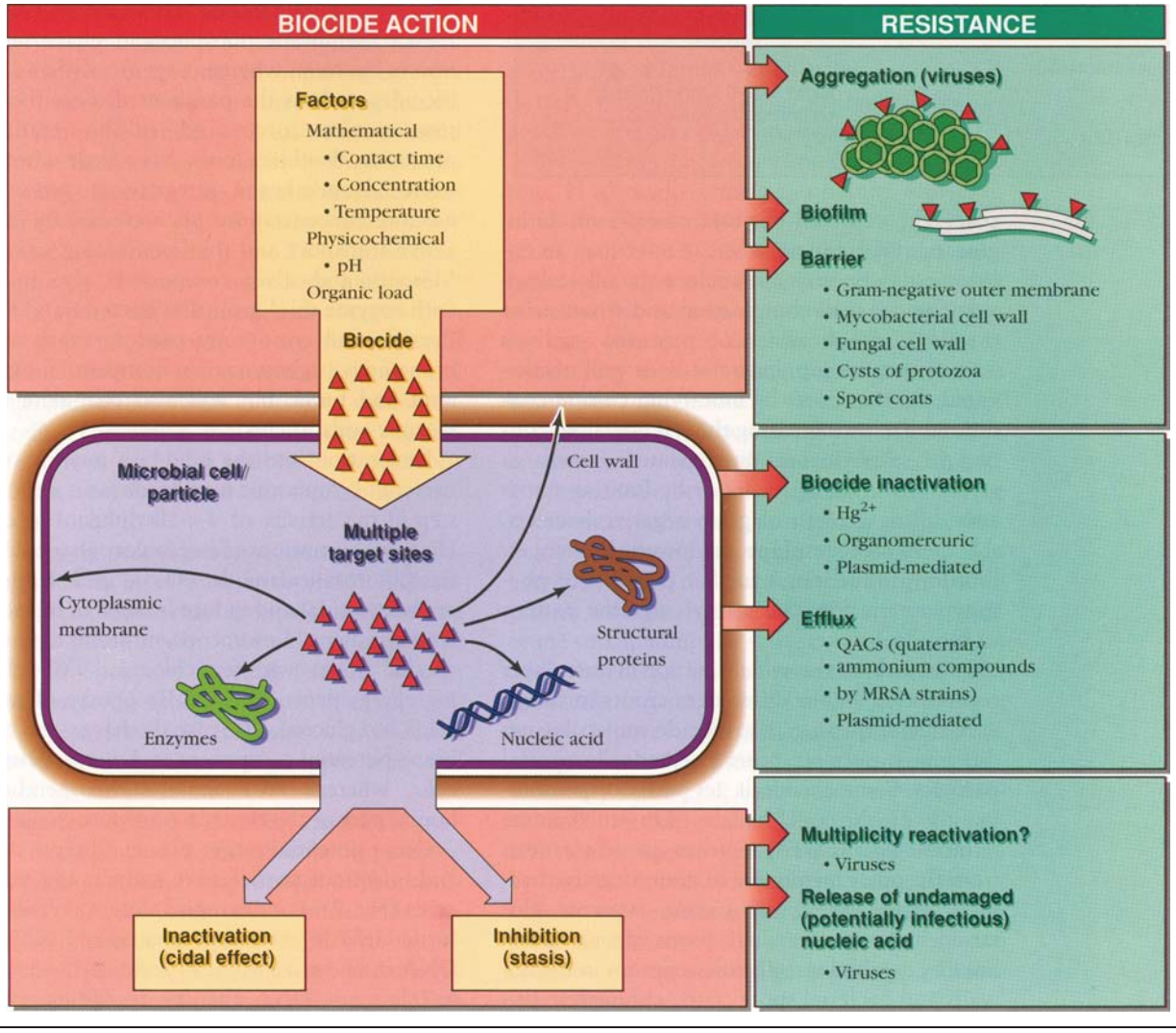

Figure 3—Microbial inactivation and resistance to biocides. Reprinted with permission from the American Society for Microbiology (*ASM News*, January 2002, p 20–24).

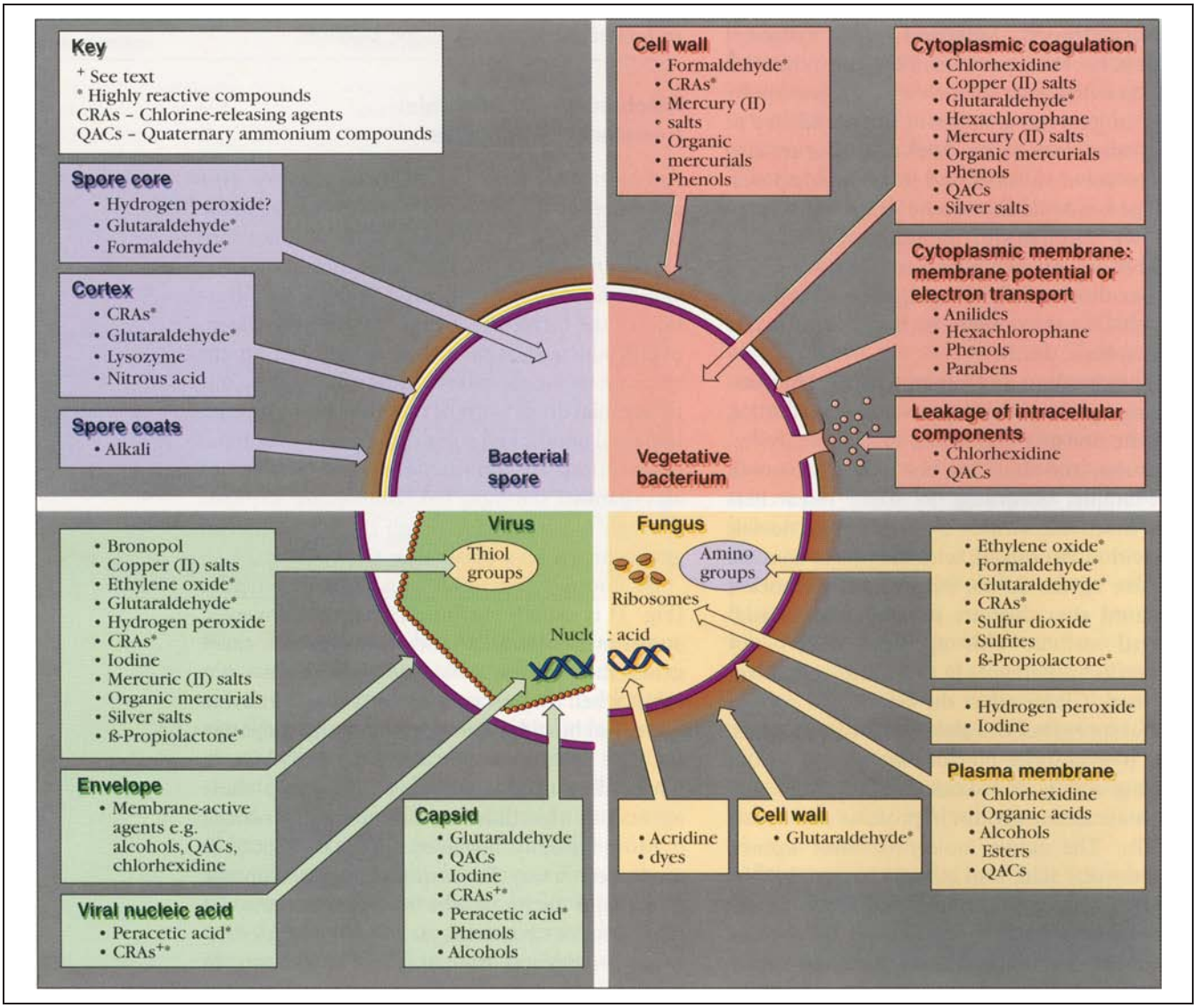

Figure 4—Mechanisms of inactivation by biocides. Thiol and amino groups in all microorganisms are susceptible to the appropriate agents shown. In vegetative bacteria and fungi, ribosomes and DNA are susceptible to hydrogen peroxide and iodine and to acridine dyes, respectively. Reprinted with permission from the American Society for Microbiology (*ASM News*, January 2002, p 20–24).

Home products

Antimicrobials are increasingly more commonplace in consumer products for home use. Levy (2001) reported that more than 700 antibacterial-containing products (for example, cleansers, soaps, toothbrushes, dishwashing detergents, hand lotions, plastic food storage containers, and bedding and bedding linens) were being marketed for the home. Other uses include food contact surfaces (cutting boards, for example), environmental surfaces, personal hygiene products, and food tissue antimicrobial sprays. Triclosan (TCS; 2,4,4′-trichloro-2′-hydroxydiphenylether), for example, has been used in skin-care products (soaps, for example) for some 30 y, and has also been used in handwashes and dental hygiene products (Russell 2004). Triclosan and parachlosulfadimethoxine/ormetoprimaxylenol (PCMX) are the most common antimicrobials used in antimicrobial hand soaps. Triclosan has also recently been incorporated into plastics such as cutting boards and knife handles, which are used in both institutional and industrial settings (Bhargava and Leonard 1996). This broad-scale use has prompted widespread concerns over the development of resistant organisms.

Human medicine

Antibiotics are used in humans in community and hospital settings primarily to treat disease, but are also used to prevent infection. The activity, action, and resistance of antiseptics and

Table 5—Naturally occurring food-related antimicrobials and sources

Antimicrobial	Source(s)	Notes
Animal-derived		
Avidin	Eggs	Binds vitamin biotin
Chitosan	Shellfish, mushrooms, fungi	Aminoglycoside; interaction with cell wall polysaccharides or cytoplasmic membrane resulting in altered permeability or transport
Conalbumin (ovotransferrin)	Eggs	Iron chelation
Lactoferrin	Milk	Iron chelation; alteration of membrane permeability; prevention of binding
Lactoperoxidase	Milk	With H_2O_2 and hypothiocyanate forms inhibitor
Lysozyme	Eggs, milk, biological secretions	Catalyzes hydrolysis of 1,4-glycosidic linkages of peptidoglycan of bacterial cell walls
Plant-derived		
Caffeine, theophylline, theobromine	Coffee, cocoa, tea	Variable activity
Flavonoids (chalcones, flavones, flavonols, flavanones, anthocyanins, isoflavonoids)	Plants	Variable activity
Humulon(e)/lupulon(e)	Hops	Some activity against Gram-positive bacteria and fungi
Isothiocyanates	*Brassicaceae* (Cruciferae)—mustard family	Allyl isothiocyanate, horseradish extract; activity may be due to enzyme inhibition
Phenolic/hydroxycinnamic acids	Plants	Caffeic, *p*-coumaric, ferulic, chlorogenic, protocatechuic, vanillic, gallic
Oleuropein	Olives	Phenolic glycoside; cytoplasmic membrane disruption
Tannins	Plants	Hydrolyzable, condensed (proanthocyanidins)
Terpenes/terpenoids	Spices	Eugenol, thymol, carvacrol, cinnamic aldehyde, vanillin, pinene, camphor, citral, borneol, thujone, menthol; interaction with the cell membrane
Thiosulfinates	*Allium* (onions, garlic)	Inhibition of sulfhydryl containing enzymes
Microbially-derived		
Bacteriocins	Lactic acid bacteria	*Lactococcus*, *Pediococcus*, *Lactobacillus*, *Leuconostoc*, *Carnobacterium* and others; bind to and form pores in cytoplasmic membrane
Natamycin	*Streptomyces natalensis*	Macrolide antifungal antibiotic; complexes with sterols in fungal cell membranes, disrupts cell membrane

disinfectants used in hospitals and other health care settings for a variety of topical and hard-surface applications were reviewed by McDonnell and Russell (1999).

Quantitative Usage Data

For the reasons addressed below, it is difficult to estimate how antibiotic usage is distributed among human, veterinary, and plant applications, and the exact amount of antibiotics introduced annually into the environment. Although the exact portion of antibiotics used in production agriculture is unknown, it is certainly significant, and likely comparable to the amount used in human medicine.

Animals

An Institute of Medicine (IOM) Committee on Human Health Risk Assessment of Using Subtherapeutic Antibiotics in Animal Feeds attempted to quantify the use of antibacterial agents in livestock and poultry feeds (IOM 1989). Using International Trade Commission (ITC) data from 1950 onward, the IOM committee estimated that in 1985 total production of antimicrobials was 31.9 million pounds. The committee noted that the reliability of the production data used in the analysis was unknown. It was estimated that 16.1 million pounds were used for disease prevention and growth promotion in animals and that 2.3 million pounds were used for disease treatment. Comparable data have not been available from the ITC since 1986, preventing updates of this estimate. Although these often-cited figures are no longer current, they provide a benchmark and demonstrate one method for quantifying antimicrobial usage.

The Union of Concerned Scientists (UCS) derived antimicrobial use estimates for cattle, swine, and poultry on the basis of drug label indications, estimates of herd size, and extent, intensity, and duration of use in each commodity or sector (Mellon and others 2001). The UCS integrated data from the USDA, National Research Council, National Animal Health Monitoring System (NAHMS), and IOM to estimate that 24.6 million pounds of antimicrobials were used for nontherapeutic uses (defined by UCS to include uses for prevention and control of disease as well as for growth promotion) in cattle, swine, and poultry in 1999. Criticisms of the UCS method of assessment included their assumptions, which were: (1) uniformity of production conditions; (2) lack of variation in use practices across producers due to product cost or personal preference; and (3) constant herd/flock size from 1984 to the late 1990s (Jones and Ricke 2003).

More recent estimates of antimicrobial usage are available from the Animal Health Institute (AHI), which estimates antibiotic use (including ionophores and arsenicals) in farm and companion

animals from data comprising responses to surveys of AHI member animal health companies. The surveys ask for the total quantity of active ingredients manufactured and sold in a calendar year by drug class, and the estimated percentage sold for the purpose of therapeutic and health maintenance (as measured by improved growth rates or more efficient feed use). AHI estimates show a general downward trend in total antibiotic use between 1999 and 2004. Production decreased from 24.9 million pounds in 1999 (of which 88.3% was for therapeutic use), to 22 million pounds in 2002 (of which 91% was for therapeutic use), and to 21.7 million pounds in 2004 (of which 95% was for therapeutic use) (AHI 2002, 2004, 2005). The AHI estimates do not include all quantities of generic antibiotics because many manufacturers of generic drugs are not AHI members. Since the majority of antimicrobials used for growth promotion are approved for other indications as well, it is difficult to determine how they were categorized by the survey respondents.

Although the AHI and UCS estimates for total use appear similar, the AHI production estimates include total animal use for all species and indications. The UCS estimates included solely nontherapeutic use in only the three major food animal species–beef cattle, swine, and broiler chickens. Thus, the UCS estimate for nontherapeutic antimicrobial use in a limited number of species is roughly 10 times the AHI estimate for all species. As noted, the UCS categorization of drugs having multiple approved uses is unclear, further complicating the interpretation of the figures. Additional points relevant to the AHI and UCS estimates are: (1) UCS used the term nontherapeutic to include prophylactic and growth promotion uses in only food animals, while AHI included growth promotion and therapeutic uses among all animals, including companion animals; (2) UCS estimated the percentage of a given food animal population that was medicated and multiplied by the product's label dosage; however, some approved products were never marketed, and others are used at varying dosage rates; (3) AHI used data provided by companies on their marketed products; other than an estimate of antimicrobials used for growth promotion, no attempt was made to further characterize usage per animal species nor to factor in the dose or duration of use; AHI did not include generic usage data; UCS may have; (4) AHI combined products into groups of antibiotics to comply with anti-trust regulations of trade associations. Therefore, despite the AHI and UCS estimates, reliable data on the amount of antibiotics used are not available, which makes assessment of effects and management difficult.

In 1999, the Alliance for the Prudent Use of Antibiotics (APUA) initiated the multidisciplinary Facts about Antibiotics in Animals and the Impact on Resistance (FAAIR) Project, which identified the critical gap in surveillance data on antimicrobial use in animals and recommended that such data be made available to improve risk assessment and better inform policy decisions on antimicrobial use in animals (FAAIR 2002). Although the World Organization for Animal Health (OIE) has proposed guidelines for the collection of quantitative antibiotic usage data, a standard method for assessing use has yet to be applied (OIE 2004). Following up on FAAIR, APUA established the Advisory Committee on Animal Antimicrobial Use Data Collection in the United States to determine the most effective means for gathering data on antimicrobial use in food animals. Comprised of varied stakeholders, from academia, government, the food animal production sector, the animal health industry, human health industry, public interest organizations, research community, and veterinarians, the committee identified methodological options for data collection. Four major categories of antimicrobial use data were identified based on the source of information and its proximity to actual use—end-user data, prescription data, manufacturing data, and distribution data.

The Advisory Committee concluded that the ideal animal antimicrobial use data collection strategy would likely combine two or more of the methods identified by the committee. Because consensus could not be reached on the ideal combination of data methods, experts comprising the committee individually rated six methodological options. The methodological options are: (1) all practices/producers record all prescriptions/use indefinitely, (2) sentinel practices/farms track use electronically, (3) selected practices/producers record all prescriptions/uses for a defined period of time, (4) periodically survey a cross-section of veterinarians/producers, (5) solicit production and sales information from manufacturers, and (6) publicly disclose production information obtained by FDA from manufacturers (DeVincent and Viola 2006).

Aquaculture

A survey conducted by the National Aquaculture Association to estimate the quantity of drugs used in the U.S. aquaculture industry indicated that only 22680 to 31750 kg of active antibiotic ingredients are sold per year (MacMillan and others 2003). Because of the small size of the U.S. aquaculture industry, and the fact that there is only one manufacturer of sulfadimethoxine/ormetoprim and one manufacturer of oxytetracycline, it is possible to accurately estimate the amount used on farms. From January 2001 to February 2003, 36126 kg of sulfadimethoxine/ormetoprim 5:1 and 22334 kg of oxytetracycline were sold for incorporation into medicated feeds for the aquaculture industry. Some minor use occurs when medicated feeds are purchased in Canada for use in U.S. salmon farms.

Plants

Fungicides are used more extensively on fruits than vegetables, with 99% of tart cherry acreage, 96% of table grape acreage, and 94% of land used for raspberry production receiving fungicidal treatment. Among vegetables, bulb onion, strawberry, and tomato led in fungicide applications, on a percent treated basis, with 87%, 86%, and 86% of acres treated, respectively (USDA 2004b). Fungicides are used much more extensively than antibiotics, with about 24000 metric tons (26000 tons) used in the United States per year.

The total amount of antibiotics used in plant agriculture has stayed fairly constant over the last decade (McManus and others 2002). In 2003, 7500 kg (16500 lb) of streptomycin were applied to about 15% of the apple and 32% of the pear acreage. Oxytetracycline use has increased from 7270 to 12270 kg (16000 to 27000 lb) between 1997 and 2003 (USDA 2004b), probably due to widespread streptomycin resistance of the target pathogens, especially on the East and West Coasts of the United States. The prevalence of imported produce necessitates an understanding of practices in the rest of the world, which in many cases are not known or reported.

Humans

Although estimates have been attempted, the quantity of human usage of antibiotics in the United States is unknown. Comprehensive estimates of total human use per annum in the United States have been reported by the AHI and UCS through their respective efforts to quantify antibiotic and antimicrobial use in food animals. AHI reported in 2000 that 32.2 million pounds of antibiotics are used annually in human medicine (AHI 2000). AHI obtained this figure indirectly by subtracting its estimate for total animal use (17.8 million pounds) from the 1989 IOM estimate of 50 million pounds (extrapolated from trends in the 1970s and 1980s to the 1990s) of use in both animals and humans (IOM

1989). The UCS estimate for human use (for inpatient and outpatient disease treatment and as topical creams, soaps, and disinfectants) was 4.5 million pounds. UCS estimates were based upon data compiled by the CDC National Center for Health Statistics (NCHS) survey of outpatient prescriptions and use, expert consultation, and a national market survey of inpatient hospital use (Mellon and others 2001).

Opportunities to acquire data on human use are greater than for animal use. In the United States, data are collected through several surveys conducted by the CDC's NCHS and the National Nosocomial Infections Surveillance (NNIS) System, comprising a collection of nosocomial (originating or taking place in a hospital) infection surveillance data from more than 300 hospitals. For the purpose of analysis, grams of antibiotics used are converted into the number of "defined daily dose(s)" (DDD) used each month in each hospital area. As defined by the World Health Organization (WHO), a DDD is an average daily dose in grams of a specific drug administered to an average adult patient (Ronning 1999). CDC also supports the collection of antibiotic use data through the Medication-Associated Adverse Event Module of the National Healthcare Safety Network (NHSN).

Private corporations are also sources of information. Under a 5-y contract established with the FDA in 2001, IMS Health (Fairfield, Conn., U.S.A.), an international corporation serving the pharmaceutical and healthcare markets with data sources from more than 29000 suppliers, has been providing market research information on drug use and the impact of pharmaceutical products on patient outcomes. The specificity and public availability of these data, however, are not yet known (IMS 2001).

Through the use of DDDs, it has been recently determined that antibiotic prescription rates within Europe vary markedly (Molstad and others 2002). In 2000, France and Germany consumed higher numbers of DDDs per capita, while the Netherlands and Denmark consumed fewer DDDs (Patrick and others 2004). In contrast to the United States, several countries, such as Denmark and Spain, have databases containing information on all antibiotics prescribed for all patients (Patrick and others 2004).

More recently, increasing attention has been given to the types of antibiotics being prescribed (Huang and Stafford 2002; Linder and Stafford 2001; Piccirillo and others 2001). Unlike the situation with animal usage, federal survey-based systems track human prescriptions and may serve as data sources for estimating use in human medicine. Antibiotic sales data are available from manufacturers, but there are limitations—sales data are not synonymous with actual consumption data, methodology is proprietary, production data are lacking.

During 2002 and 2003, penicillins were the most prescribed class of antibiotics in hospital outpatient and physician office visits in the United States (HHS/CDC/NCHS 2005). The number of antibiotic prescriptions in adults and children in U.S. ambulatory care settings declined from 151 million to 126 million between 1992 and 2000 (McCaig and others 2003). Also documented during this time period was evidence of increasing outpatient use of amoxicillin and cephalosporins (Steinman and others 2003). The 34% decrease in the rate of prescriptions written for children during physician office visits, and lack of increase for adults during a 20-y span may suggest that the efforts of the CDC, medical associations, and other stakeholder groups may be having a beneficial effect on prudent antibiotic use and overall prescription writing.

Factors contributing to the overuse of antibiotics in humans include real or perceived pressure from adult patients and parents of child patients to prescribe antibiotics, inadequate identification of label indications for some drugs, lack of awareness of prescription guidelines, the move toward managed healthcare, and inadequate time for physicians to explain to patients that antibiotics are often unnecessary (Hutchinson and Foley 1999; Okeke and others 1999; WHO 2002). A Congressional Research Service report noted that 96% of pediatricians surveyed reported that parents of children in office visits specifically requested an antibiotic prescription, and 33% prescribed an antibiotic without a clinical basis simply to appease the parent (Vogt and Jackson 2001). Hamm and others (1996) stated that parents and patients perceive that "they haven't gotten their money's worth" in appointments with primary care physicians that do not result in a prescription being written. Additionally, Avorn and Solomon (2000) pointed out that the number of patients seen per hour by physicians is increasing due to increasing administrative demands and that writing a prescription can serve as a termination strategy for an office visit.

Mechanisms for Emergence and Dissemination of Antimicrobial Resistance

Emergence

As pointed out by Courvalin (2005), resistance to antimicrobial drugs is an unavoidable aspect of the general evolution of bacteria that occurs by chance. Mechanisms for emergence of bacterial resistance are quite diverse as are the modes of action of antimicrobials, which may include inhibition of various steps of DNA replication, transcription, and translation, or action at the level of the cell wall or cell membrane.

Microbial strategies for resisting the effects of antibiotics include impaired uptake, modification or overproduction of the target sites of antimicrobials, bypass of sensitive steps, absence of enzymes or metabolic pathways, and efflux of the antimicrobial drug (Russell and others 1997). Further, bacteria can resist the effects of antimicrobials by enzymatically degrading the drug before it reaches its target site, altering the protein(s) within the bacterium that serve as receptors for the antimicrobials, and changing their membrane permeability to the antibiotics (Cloete 2003; Dever and Dermody 1991).

Efflux pumps. Called "multidrug efflux pumps," these systems for transporting substances out of cells often provide resistance to a variety of structurally different antimicrobials, including antibiotics, dyes, and surfactants. Along with impaired uptake, efflux pumps are a main strategy that bacteria use to deal with the stress of sanitizer exposure (Russell and others 1997). Gram-positive and Gram-negative bacteria use the same efflux system for ethidium bromide and QACs. Tetracycline resistance in *E. coli* is at least partially due to an energy-dependent efflux mechanism (McMurry 1980), and a similar mechanism has been implicated in *E. coli* fluoroquinolone resistance (Cohen and others 1988, 1989; Hooper and others 1989). In addition, the genes for multiple antibiotic resistance in *Pseudomonas aeruginosa* may be on an efflux operon[11] (Poole and others 1993). Efflux mechanisms, however, do not pertain to bacteriocins, which do not accumulate intracellularly.

Acid tolerance can be viewed in terms of efflux ability. The mechanism by which organic acids inhibit microorganisms involves passage of the undissociated form of the acid across the cell membrane lipid bilayer. Once inside the cell, the acid dissociates because the cell interior has a higher pH than the exterior. Protons generated from intracellular dissociation of the organic acid

[11] operon: chromosome segment having an operator gene and the closely linked structural gene or genes whose action it controls (IFH 2000).

acidify the cytoplasm and must be extruded to the exterior. Yeasts develop resistance to sorbic and other organic acids via several mechanisms. They use the enzyme H^+-ATPase along with ATP (adenosine triphosphate) energy to remove excess protons from the cell. Inhibition and/or inactivation of the yeasts may be due to eventual loss of cellular energy or inactivation of critical cellular functions due to low intracellular pH. To prevent energy depletion, a membrane protein may be induced for decreasing ATPase activity and thus conserve energy (Brul and Coote 1999). Exposure of *Saccharomyces cerevisiae* to sorbic acid induces a multidrug resistance pump (membrane protein ATP-binding cassette transporter Pdr12 [Holyoak and others 1999; Piper and others 1998]), which confers resistance by mediating energy-dependent anion extrusion (Piper and others 1998). To circumvent the problem of extruded anions and protons reentering the cell upon recombining in the extracellular medium, adapted yeasts apparently reduce diffusion of the weak acids, most likely by altering cell membrane structures to reduce passage of the acids into the cell (Brul and Coote 1999). Similar mechanisms likely also exist for bacteria capable of developing resistance to sorbic or other organic acids.

Enzymatic degradation. A common phenomenon, enzymatic degradation, is the primary mechanism of resistance to β-lactam antibiotics via the hydrolysis of the β-lactam ring (Bush and Sykes 1984) and the resistance mechanism for chloramphenicol and aminoglycosides. Resistance to chloramphenicol, a broad spectrum antimicrobial, occurs through acetylation catalyzed by chloramphenicol acetyltransferase; other modes of resistance are also possible, however (Dever and Dermody 1991; Kucers and Bennett 1987). Methylases, acetyltransferases, nucleotidyltransferases, and phosphotransferaces are used against aminoglycosides (Davies 1994; Shaw and others 1993). Enzymic degradation of food antimicrobial agents can be specialized or general, but would be different from the enzymes that inactivate antibiotics. For example, some bacteria metabolize citric acid, rendering it ineffective against them. In contrast, many proteases inactivate bacteriocins in a nonspecific fashion. A nisin dehydroreductase conveys resistance by inactivating a nisin dehydro residue (Jarvis and Farr 1971).

Alteration of receptors. Alteration of specific receptor sites prevents proper target recognition. Resistance to nalidixic acid is most often due to mutations in *gyr*A and *gyr*B, the genes encoding the target proteins of the antibiotic. Resistance to ciprofloxacin is also associated with mutations in gyrA and gyrB (Heddle and Maxwell 2002; Hooper 1995; Tankovic and others 1996).

Membrane permeability change. The most common form of intrinsic resistance to antibiotics is due to membrane structure and composition, which can naturally act as a permeability barrier or undergo change through acquired resistance mechanisms, as in the case of Gram-negative bacteria. *E. coli* resistance to β-lactam antibiotics, for example, occurs upon replacement of the outer membrane OmpF porin by the narrower OmpC porin (Nikaido and others 1983) and in *Staphylococcus epidermidis* glycopeptide resistance may occur through over production of glycopeptide binding sites within the cell wall peptidoglycan (Sanyal and Greenwood 1993). Resistance to nisin can result from spontaneous genetic mutation (designated Nis^m) involving bacteriocin adsorption or membrane insertion, presumably causing loss of cell membrane fluidity and hindering nisin insertion (Nis^m cell membranes are more solid than those of the wild-type strain).

Membrane fluidity can play an important role in resistance of *L. monocytogenes* to antimicrobials (Juneja and Davidson 1993). *L. monocytogenes* cells grown in the presence of C14:0 or C18:0 fatty acids have higher phase transition (T_c) and increased resistance to four common antimicrobials than cells grown in the presence of C18:1, which have lower T_c and are more sensitive. It is assumed that the higher phase transition temperature of the membrane fatty acids prevents effective penetration of the pore-forming bacteriocin. Nisin-resistant *C. botulinum* also have altered membrane fatty acid composition that would increase their membrane rigidity (Mazzotta and Montville 1999).

Stress-adaptation, co-selection, cross-resistance, and cross-protection

Mechanisms exist whereby microorganisms that are resistant to one antimicrobial may become resistant to others (Yousef and Juneja 2003). Exposure to subinhibitory concentrations of an antimicrobial, for example, may activate intrinsic resistance mechanisms, thereby decreasing susceptibility of the microbe to the inducing agent and in tandem decreasing susceptibility to other, unrelated antimicrobials. In other instances, resistance to several antimicrobials having unrelated targets or modes of action may result from co-selection, which involves sequential linking of separate genes conferring resistance to different antibiotics, often on plasmids or integrons,[12] and transferred together. Cross-resistance is the occurrence of resistance to antimicrobials because they have the same molecular targets. Cross-protection occurs when adaptation to one stress is associated with increased resistance to another, unrelated stress. Correlations among these mechanisms are seen in some cases, but the root causes of the dissemination of the resistance remain unknown.

Strains of *E. coli* resistant to thymol and eugenol (essential oils found in thyme and cloves, respectively) were found to be more resistant to chloramphenicol (Walsh and others 2003). Because stable resistance to the essential oil components was not readily detected, the authors denoted the increased resistance as "tolerance" (Walsh and others 2003). In contrast, methicillin-resistant *Staphylococcus aureus*, however, were found to be as sensitive to oregano essential oil and its components, carvacrol and eugenol, as methicillin-sensitive strains (Nostro and others 2004). Resistance to carvacrol, however, which is associated with changes in the cellular membrane, apparently does not confer resistance to other membrane-active compounds. *Bacillus cereus* adapted to carvacrol were demonstrated to be more sensitive to subsequent nisin exposure than nonadapted cells (Pol and others 2001).

Bacteria are able to produce stress response proteins when subjected to subinhibitory levels of stress (Yousef and Juneja 2003). A variety of situations can induce transcription and translation of stress response proteins, which convey increased resistance to a multitude of stressors. For example, exposure of *E. faecalis* to subinhibitory levels of sodium chloride, sodium dodecyl sulfate, and bile salts conferred a protective effect against heat compared to nontreated cells (Flahaut and others 1997). Heat shock proteins (HSP) comprise one of the most well-studied classes of stress response proteins, although the HSP levels do not correlate with the extent or persistence of protection (Jorgensen and others 1996; Mackey and Derrick 1990). HSP are typically regulated by sigma factors such as RpoS or RpoH, which are subunits of RNA polymerase.

Salmonella enterica serovar Enteritidis and *L. monocytogenes* first exposed to alkali are more resistant to heat treatment than those not pre-exposed (Humphrey and others 1991; Taormina

[12] integrons: genetic elements that capture and link multiple drug resistance genes together into a single locus

and Beuchat 2001). Studies with *Salmonella* Enteritidis showed that treatment with low levels of alkali (pH 10.0 sodium hydroxide or trisodium phosphate) resulted in a decrease in protein expression of 15% and 22%, respectively (Sampathkumar and others 2004). Some outer membrane proteins, identified as protein chaperones and housekeeping proteins involved in biosynthesis, were up-regulated. Similarly, when *E. coli* K-12 was shifted from pH 7 to 8.8, known HSPs were induced (Taglicht and others 1987).

Hong and others (2002) found that *Streptomyces coelicolor*, containing a plasmid encoding a signal transduction system including the sigma factor E (Φ^E), demonstrated lysozyme-induced resistance to kanamycin (100g/mL). *L. monocytogenes* has been shown to contain a similar signal transduction system (CesRK) that is activated upon introduction of lysozyme to the cells and results in antibiotic resistance.

An example of an intrinsic resistance system is the multiple antimicrobial resistance (mar) operon, a global regulator that controls intrinsic resistance to unrelated antibiotics and other cytotoxic substances (Alekshun and Levy 1999). Golding and Matthews (2004) demonstrated decreased susceptibility of *E. coli* O157:H7 to multiple antimicrobials, putatively linked to a mutation in the mar operon, following exposure to chloramphenicol. Potenski and others (2003) found that upon treating *Salmonella* Enteritidis cells with sublethal levels of chlorine, sodium nitrite, sodium benzoate, or acetic acid, the cells exhibited resistance to tetracycline, chloramphenicol, nalidixic acid, and ciprofloxacin, thus determining that a mar operon was responsible for the resistance responses.

Antimicrobial resistant phenotypes of *E. coli* O157:H7 may also be related to acquisition of class 1 integrons (Zhao and others 2001a), which is significant because the integrons may contain several antimicrobial gene cassettes and, therefore, co-select for resistance to other antimicrobials.

Genes encoding for multidrug efflux systems in *S. aureus* have been located on plasmids (generally 18 to 57 kb in size) also containing genes for resistance against penicillin, gentamicin, trimethoprim and kanamycin (Lyon and others 1984). The *qac*A and *qac*B genes have been found on plasmids that also confer resistance to various antibiotics, including penicillin (Lyon and Skurray 1987). Twenty-four QAC-resistant *Staphylococcus* isolates were analyzed for resistance to selected antibiotics and dyes (Heir and others 1999). Five of the seven strains with the QAC resistance genes *qac*A/*qac*B had high-level resistance to penicillin G and ampicillin. One isolate containing the *smr* gene showed resistance to ampicillin, penicillin G, tetracycline, erythromycin, and trimethoprim, but not to chloramphenicol, gentamicin, norfloxacin, kanamycin, or vancomycin. It was suggested that the antibiotic resistance in this strain was due to resistance markers on the chromosome or other plasmids harbored by the strain. All other sanitizer-resistant isolates were generally susceptible to antibiotics.

Several studies have found a lack of cross-resistance between agents, even when mechanisms appear similar. For example, when acquired resistance mechanisms for biocides, which can closely resemble those for antibiotics, were studied by Aase and others (2000), no connection was found between QAC resistance and antibiotic resistance in *L. monocytogenes*. They evaluated 200 *L. monocytogenes* isolates from various food, human, and environmental sources from Norway and Europe and found that 10% were resistant to benzalkonium chloride (BC), while none of the isolates was resistant to any of the 15 antibiotics. Both resistant and sensitive strains responded approximately equally to BC after adaptation, and remained stable during subculturing in the absence of BC. They suggested that genes coding for the efflux pumps providing resistance against QAC and ethidium bromide are not located on the multiple drug resistance (MDR) plasmid. When sublethal levels of a triclosan-containing domestic detergent were applied to a biofilm, the composition of the biofilm changed; however, the remaining organisms were generally as susceptible to a host of antibiotics and other antimicrobials as the initial population (McBain and others 2003).

There are many other instances where resistance to one antimicrobial does not confer resistance to another. This can often be explained by a mechanistic understanding of the agent's effect on the cell. Enterococci are particularly resistant to heat and sodium hypochlorite (Freeman and others 1994; Kearns and others 1995), which may permit their survival of intervention techniques in both food processing and clinical settings. In one study, vancomycin-resistant enterococci did not have enhanced resistance to chemical disinfectants or to heat (Bradley and Fraise 1996). This was confirmed by Panagea and Chadwick (1996), who found no differences in heat tolerance of vancomycin-resistant or sensitive clinical isolates of *E. faecium*.

Bertolatti and others (2001) and Walsh and others (2001a) reported that the potential for antibiotic-resistant organisms to exhibit enhanced resistance to food preservation techniques or food antimicrobial agents has been studied only to a limited extent. Antibiotic-resistant Gram-positive cocci and streptomycin-resistant *L. monocytogenes* responded similarly to heat compared with the corresponding wild-type strains. Other investigations have considered the decimal reduction times (*D*-values) of antibiotic-resistant organisms with or without induced acid tolerance to determine whether heat resistance is altered in the strains. For example, Bacon and others (2003a) examined thermal *D*-values of wild type and MDR-*Salmonella* strains isolated from bovine sources and grown in various levels of glucose to stimulate an acid tolerance response (ATR). At 59 °C, acid-tolerant cultures had increased thermal resistance compared with nonacid tolerant controls. The D_{61° C values of antimicrobial susceptible *Salmonella* strains increased as the glucose concentration (acid tolerance) in the culturing medium increased, but D_{61° C values of MDR-cultures were similar, irrespective of ATR. When averaged across glucose levels and temperatures, *D*-values of antimicrobial susceptible and resistant *Salmonella* cultures were similar. The results suggest a cross-protective effect of acid adaptation on thermal inactivation, but no association between antimicrobial susceptibility and heat resistance. When the ATR was induced in either type of strain by growth in glucose, some strain variations in acid resistance were observed, but no association between susceptibility to antimicrobial agents and potential to survive a low pH stress was made (Bacon and others 2003b). Lopes (1998) reported observing that antibiotic-resistant strains of *Salmonella* Typhimurium and *L. monocytogenes* were equally as susceptible to sanitizer treatments as antibiotic sensitive strains. They found that *Salmonella* Typhimurium strains resistant to nalidixic acid and *L. monocytogenes* strains carrying plasmid pGK12 encoding resistance to chloramphenicol, erythromycin, and rifampin did not exhibit resistance to organic acid/anionic surfactant-based sanitizers. Others have concluded that in *Listeria* spp., including the pathogenic *L. monocytogenes*, plasmid-mediated disinfectant resistance may not necessarily be linked to antibiotic resistance (Lemaitre and others 1998).

Mazzotta and others (2000) found that nisin-resistant *L. monocytogenes* and *C. botulinum* were not more sensitive to food preservatives such as low pH, salt, sodium nitrite, and potassium sorbate. Hossack and others (1983), however, reported that nisin resistance in *S. aureus* was linked with antibiotic resistance, observing that antibiotic MICs increased as much as 30-fold among

the nisin-resistant strains. Several studies suggest that nisin resistance results in physiological changes that decrease resistance to other agents. These data are not necessarily inconsistent, as different antibiotics have different modes of action, which may or may not be affected by the changes in membranes. Szybalski (1953) reported that a penicillin-resistant *S. aureus* mutant was 50 times more sensitive to nisin. Severina and others (1998), however, found that several MDR-bacteria remained sensitive to nisin treatment. Similarly, studies with nisin-resistant *L. monocytogenes* or cells pretreated with nisin showed no significant increase in resistance to antibiotics (Crandall and Montville 1998). McEntire (2003) observed that the nisin-resistant strain was highly sensitive to second and third generation cephalosporins, at concentrations where the wild type was virtually unaffected. The mutant also exhibited increased acid sensitivity due to increased ATPase activity; while acid sensitivity may not be directly related to nisin resistance, both phenotypes may be directly or indirectly controlled by the same signal transduction system (Cotter and others 2002; McEntire and others 2004).

"Collateral sensitivity" (a mutation or adaptation conferring resistance to one or more agents which simultaneously increase sensitivity to other agents) is not unique to nisin resistance. *Bacillus licheniformis,* which is resistant to the bacitracin it produces, is highly sensitive to detergents, likely due to a specific membrane change (Podlesek and others 2000). After exposure to alkali cleaning solutions, 4 of 5 strains of *L. monocytogenes* were as sensitive or more sensitive to heat than unexposed cells, and all were more sensitive to the sanitizer components (free chlorine, benzalkonium chloride [BC], and cetylpyridinium chloride) compared with the controls (Taormina and Beuchat 2002).

Development of resistance to acid and heat among pathogens may influence their behavior when exposed to fermentation, drying, cooking, or consumption in the human host. The increased virulence may stem from the influence of acid resistance on microbial behavior upon exposure to the final barrier (gastric secretions, phagocytosomal vacuoles) in the human host. Thus, in addition to increased resistance against food preservation treatments, stress-adaptation may lead to increased virulence and lower infectious doses (Samelis and Sofos 2003a).

Stopforth and others (2004a), however, indicated that similarly acid-adapted (glucose) *E. coli* O157: H7 inocula were not different than controls in survival when inoculated in wounds of apples and exposed to water or sanitizing solutions of acetic acid, hydrogen peroxide, and sodium hypochlorite. Ikeda and others (2003) found no differences in survival or growth of acid-adapted (glucose) *L. monocytogenes* inocula on fresh beef decontaminated with hot water and organic acid solutions. Calicioglu and others (2002a, 2002b, 2003a, 2003b, 2003c, 2003d) reported that inactivation of acid-adapted (glucose) inocula during drying and storage of beef jerky was more efficient than that of normal cultures (grown in broth without glucose), potentially indicating that exhaustion or stressing of the cells during acid adaptation caused the cultures to be more sensitive to the subsequent stresses of acid, heat, and dehydration, and confirming the importance of the hurdle concept in food preservation.

As is the case for other pathogens, *L. monocytogenes,* which can grow at a pH as low as 4.39 (George and others 1988), can exhibit the ATR with increased survival of prestressed cells at normally lethal acid levels (Bonnet and Montville 2005; Gahan and others 1996; Samelis and others 2003). This adaptive mechanism, which may occur in different pH ranges for different microorganisms (Koutsoumanis and Sofos 2004b), does not enhance the ability of the organism to grow, but has several implications for food safety due to the increased pathogen survival rates. For example, Bonnet and Montville (2005) showed that ATR-induced *L. monocytogenes* coinoculated in broth with a nisin-producing *Lactococcus lactis* persisted in the majority of samples for at least 30 days. *L. monocytogenes* that were not induced to ATR, however, could not be detected. Cross-protection of acid tolerance in *L. monocytogenes* with thermal tolerance, crystal violet, ethanol, and osmotic stress has also been demonstrated (O'Driscoll and others 1996).

Since *L. monocytogenes* must be able to bypass the acidity of the stomach in order to be infective, the impact of ATR induction on microbial survival with preexposure to acids or other stressing antimicrobial hurdles in simulated gastric fluid has been of interest. Results indicate that simulated gastric fluid acid tolerance may depend on type and composition of product, microbial cell concentration, cell age, and product storage time (Stopforth and others 2005). The authors noted observing, for example, higher gastric fluid ATR with increased product fat content. However, spontaneous mutants of *L. monocytogenes* with constitutive acid tolerance showed increased virulence in mice when administered intraperitoneally, suggesting that a mechanism in addition to gastric acid resistance is involved (O'Driscoll and others 1996).

Dissemination of resistance determinants between microorganisms

Two main factors contribute to the persistence of antimicrobial resistant microorganisms in the environment: survival of the microorganism and maintenance of the resistant genotype. Dissemination of resistance determinants can occur at three levels—bacterial (clonal spread), replicon (plasmid epidemics), or gene (transposons), all three of which coexist in nature and are not only infectious but exponential as well, since all are associated with DNA duplication (Courvalin 2005).

The extent to which dissemination and transfer of antimicrobial resistance determinants occur in nature is not well understood, but many suggest that antimicrobial resistance genes are widely disseminated in nature (Riesenfeld and others 2004; Sundin 2002) and present in a diversity of microorganisms and niches (Chee-Sanford and others 2001; Nield and others 2001; Riesenfeld and others 2004). Further, the same genes are present in a diversity of bacteria, including evolutionary disparate microorganisms (for example, Gram-negative in contrast to Gram-positive bacteria [LeBlanc and others 1988; Werner and others 2001]) and bacteria from different environments (Bolton and others 1999; Sanchez and others 2002).

The "mobility" of these antibiotic resistance genes is attributed to their residence on mobile genetic elements—plasmids (Navarro and others 2001; Smalla and others 2000), transposons (Sundin 2002), and integrons (Nandi and others 2004), described in detail in Appendix 2. Gene transfer between pathogens is not a new concern and has been reported in pathogens of both humans and animals. Although the existence of mobile genetic elements predates the widespread use of antibiotics (Hughes and Datta 1983), current problems have arisen because more and more resistance genes have become linked in multiple, tandem repeats in these mobile DNA elements.

Starliper and others (1998) examined strains of *E. ictaluri* resistant to sulfadimethoxine/ormetoprim and found that resistance to sulfadimethoxine/ormetoprim and tetracycline was carried on a 55 kb R-plasmid. The R-plasmid allowed very fast and efficient transfer of resistance between *E. ictaluri* and *E. coli* and vice versa. Although the origin of the plasmid was unknown, it was found to be essentially identical to a plasmid found in

Tribrissen®-resistant *E. coli* strain 1898 originating from a case of equine cystitis (Cooper and others 1993). The implication was that antibiotic resistance found in the fish pathogen could possibly have originated with bacteria colonizing warm-blooded animals.

Chee-Sanford and others (2001), possibly the first group to use DNA technology to study the genes for a major class of antibiotic resistance in groundwater potentially impacted by animal agriculture, used PCR typing methods to assess the presence of tetracycline resistance determinants in waste lagoons and groundwater underlying two swine farms impacted by waste seepage. All eight classes of genes (*tet*(O), *tet*(Q), *tet*(W), *tet*(M), tetß(P), *tet*(S), *tet*(T), and *otr*(A) encoding this mechanism of resistance were found in total DNA extracted from water from both lagoons. The authors noted that the maximal relative frequency and diversity of tetracycline resistance genes occurred at waste lagoons and gradually declined in the direction of groundwater flow; however, one of the genes was still detectable 250 meters downstream.

Agerso and others (2004) studied the presence of the *tet*(M) gene in farmland soil by direct detection of the gene. They reported that the gene was most prevalent in farmland soil immediately after spread of pig manure slurry, but could be detected on farmland soil 2 y after the field had been treated. On soil not treated with animal manure, *tet*(M) could only be detected after selective enrichment with tetracycline present in the media under anaerobic and aerobic conditions. The results indicate that the *tet*(M) gene is spread with bacteria in the manure, but that it is also present in the indigenous soil microflora, possibly occurring specifically in the facultative anaerobic bacteria. Sengelov and others (2003) investigated the level of tetracycline, erythromycin, and streptomycin resistance among bacteria before and after spread of pig manure slurry on fields. They found that the ratio of colony forming units (CFU) of tetracycline-resistant bacteria to all bacteria was significantly higher immediately after spread of pig manure slurry. The ratio decreased rapidly 1 y after the spread, showing no accumulation of tetracycline-resistant bacteria. No effect on erythromycin- and streptomycin-resistant bacteria in farmland soil was observed in the study.

Another means of environmental transfer of antibiotic resistance genes from the antibiotic-producing strain might be through direct ingestion of medicated feeds by food animals. It has been shown that a DNA-encoding homolog of the *van* resistance gene cluster was a contaminant of feed-grade avoparcin. Thus, it was proposed that the ingested glycopeptide resistance gene complex was present, conferred resistance to this antibiotic, and in the presence of the selective pressure of the glycopeptide avoparcin in the food animal, selected for increased numbers of resistant strains (Lu and others 2004; Marshall and others 1998). However, another study based on amino acid sequence homology showed that horizontal transfer to human or animal bacteria of antibiotic resistance genes from bacteria that are used for antibiotic production was unlikely (Lau and others 2004).

Transfer to humans from various sources

Data on the transfer of resistant organisms from animal or environmental microbial isolates to humans, ability to cause illness, and resultant treatment failure are valuable for assessing the overall impact of antimicrobial resistance on human health. The transfer of antimicrobial-resistant bacteria from food animals to humans is well documented (Sanchez and others 2002; Swartz 2002). Evidence includes transfer of *Salmonella* from cattle, chickens, pigs, and turkeys (Angulo and others 2000; Mead and others 1999; Meng and others 1998) and *Campylobacter* species from chickens and turkeys in a commercial operation (Altekruse and others 2002). Farmers may be at a greater occupational risk of acquiring antimicrobial resistant bacteria from the environment. A range of microorganisms including *S. aureus*, nongroupable *Streptococci, Enterobacter, Enterococci*, and *E. coli* isolated from farm workers was significantly more resistant to most antimicrobials than isolates from nonfarm workers (Aubry-Damon and others 2004).

Most ceftriaxone-resistant *Salmonella* infections are acquired outside the United States. A domestically acquired ceftriaxone-resistant *Salmonella* infection, however, was reported in a 12-year-old child (Fey and others 2000; Herikstad and others 1997). The ceftriaxone-resistant *Salmonella enterica* isolate that infected the child was indistinguishable from one of the ceftriaxone-resistant isolates present in a herd of cattle during an outbreak on the family ranch. Although use of ceftriaxone or other antimicrobials in the herd could not be established, it was suggested that use in the herd of ceftiofur, a broad-spectrum cephalosporin approved for use in cattle, most likely led to the emergence of resistance in the *S. enterica* in these cattle, transmission of the resistant strain from the cattle to the child, and illness in the child (Fey and others 2000). The means of transmission of the ceftriaxone-resistant *Salmonella* from the cattle to the child was not known; however, Fey and others (2000) thought it unlikely that the child's infection was actually foodborne. They concluded that the inoculum of *Salmonella* necessary to cause illness in the child might have been lowered by the prior treatment of the child with amoxicillin-clavulanate and ampicillin-sulbactam.

Resistant bacteria on food crops destined for consumption by humans may provide a route of delivery of resistance genes to the human intestinal flora. *Enterobacteriaceae* are not only found in abundance in the environment, but as pathogens as well as commensals in the human gastrointestinal tract. For example, the same serotypes of *E. coli* and *Klebsiella* were found in food served in a hospital setting and isolates of consuming patients (Cooke and others 1970; Cooke and others 1980). A Finnish study investigated the potential for raw vegetables to serve as a source of resistant strains of *Enterobacteriaceae* (Osterblad and others 1999); researchers concluded that bacteria from vegetables were not responsible for the high prevalence of resistant *Enterobacteriaceae* in fecal flora. More research is warranted to determine the impact of antimicrobial resistant environmental commensal bacteria as an important source of resistance in fecal flora.

Cross-species infections between plants and humans are increasingly recognized (Tan 2002; Vidaver 2005). *Pseudomonas aeruginosa, Burkholderia (Pseudomonas) cepacia*, and *Serratia marcescens*, which can be plant pathogens, are potential serious human pathogens. The plant pathogens are intrinsically antibiotic resistant (Vidaver 2005). However, as yet, there are no data that indicate transfer of antibiotic resistance determinants from the plant pathogens to bacteria causing human disease, or vice versa, under natural conditions (McManus and others 2002).

Three hundred species of fungi have been reported as causing cutaneous and invasive human infections (Taylor and others 2001). The level of invasive infections is attributed in part to increased organ transplants and attendant immunosuppression, as well as complications arising from AIDS, although fungal diseases are reported in "normal individuals" as well (Ponton and others 2000). The human health concern is that some of the bacteria and many of the fungal taxa long known as plant pathogens are being isolated from human infections (Vidaver 2005).

The significance of antibiotic use in domestic aquaculture to food safety and human health is unknown. Ultimately, data

relating to the persistence of antibiotic residues and bioactivity in the fish farm environment and the ability of fish pathogens to transmit antibiotic resistance determinants to human pathogens will be required. Most fish pathogens do not infect humans because they are incapable of growing at human body temperatures; thus, the risk of transmission of pathogens from fish to humans is very small. So far, the potential seems more likely for human or animal pathogens to transmit resistance to fish pathogens. Currently, antibiotic usage in aquaculture is at its lowest point since the early 1980s, and until new drugs are approved, the situation seems unlikely to change.

Detection of Resistance

Resistance among microorganisms can generally be detected either phenotypically or genotypically. For clinically important bacteria, diagnostic laboratories perform phenotypic-based analyses using standardized susceptibility testing methods, usually in accordance with those published by the Clinical and Laboratory Standards Institute (CLSI, www.clsi.org, Wayne, Penn., U.S.A.). Determination of resistance via phenotype uses growth inhibition assays performed in broth or by agar disc diffusion. In a dilution-based growth inhibition assay, the minimal inhibitory concentration (MIC) can be calculated for each bacterial isolate and antimicrobial drug, and then interpreted as either susceptible, intermediate, or resistant. This type of assay enables the practitioner to more readily choose the antibiotic that is most appropriate for clinical use because a susceptible interpretation conveys likely favorable clinical outcome, whereas resistant conveys likely treatment failure.

These MIC category "breakpoints" are based on an evaluation of the clinical efficacy of the drug, its pharmacokinetics and pharmacodynamics, and a comparison of MICs of microorganisms from a variety of sources. Although a "high" MIC might indicate that a given pathogen has a genetically based resistance mechanism, this is not necessarily the case, since the breakpoint is set, in part, on the basis of achievable drug concentrations at the site of the infection. If the MIC is greater than the needed concentration, or does not meet certain other pharmacokinetic parameters, then the pathogen can be considered resistant, regardless of resistance mechanism. CLSI has established antimicrobial susceptibility testing methods for animal and human pathogens, and breakpoints for many microbes and drugs. Currently, no standard methods are routinely used in clinical laboratories for determining genotypic resistance and predicting clinical outcomes.

Identifying resistance versus susceptibility to food antimicrobial agents and/or sanitizers may be problematic because there are no standardized testing methods or accepted breakpoint values for these substances (Chapman 1998; Parish and Davidson 1993). An important caveat to most studies of biocide resistance, however, is that resistance is based on comparison of MICs among bacterial strains, wherein strains are generally characterized as resistant if MICs are 4- to 10-fold higher than for sensitive strains. Of note, the effective use concentrations of QACs and other sanitizers are much higher than MIC values denoting resistance. For example, if the MIC of an agent is 4 units/mL, and a strain survives 20 units/mL, it may be termed resistant. However, the standard concentration of the QAC or sanitizer used may be 1000 units/mL, making the observed "resistance" irrelevant.

The phenotypic approach, involving cultivation (culturing) of bacteria and testing them against antibiotics, is the traditional method of detecting resistance among bacteria from water or soil, but is problematic for this application for a number of reasons. Bacterial isolation techniques are often highly selective and may miss the majority of bacteria in a sample that are not the study target and the less predominant strains. These techniques will also miss the bacteria that cannot grow in the laboratory. The vast majority of intestinal bacteria that contaminate the environment live as commensals and typically do not grow in laboratory conditions. A culture-independent approach, however, which analyzes the total DNA extracted from a sample for presence of resistance genes, is a suitably sensitive approach. Molecular detection techniques, such as polymerase chain reaction or DNA–DNA hybridization, are standard techniques used to determine the presence of specific resistance genes. Microarrays[13] have been used to test for the presence of a number of genes from a given bacterial isolate (Call and others 2003; Yu and others 2004).

Monitoring of Resistance

Monitoring systems

Several countries and communities have surveillance programs in place that measure resistance trends over time. Interpretation and comparisons between country systems and surveys are often hampered by lack of standardized methods, differences in methodology, and lack of validated interpretive criteria. Also, review of the literature is hampered by lack of continuity between studies. Many studies have reported susceptibility data determined from the disk diffusion method, but differences among specific techniques in the disk diffusion method do not allow valid comparisons among studies. Other studies reported susceptibility data obtained from serial broth dilution or MIC testing. Harmonization of methods between these programs must occur before international comparisons can be made and international resistance trends elucidated.

Resistance to antimicrobials includes fungi. Because resistance to fungicidal agents is relatively common in agriculture, a global monitoring system—the Fungicide Resistance Action Committee (www.frac.info)—has been established. There is no fungicide resistance monitoring system counterpart for human health yet, although the incidence of invasive fungal infections in humans is a growing concern.

United States

The principal domestic system for monitoring antibiotic resistance of food-related bacteria is the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS), established in 1996 as a collaborative effort among the Centers for Disease Control (CDC), FDA, and USDA. The surveillance system was initiated to monitor changes in susceptibilities of zoonotic pathogens in animals, animal products, and humans.

Bacterial isolates are collected from human and animal clinical specimens, healthy farm animals, and raw foods of animal origin. The isolates are tested to determine MICs for selected important antimicrobial classes used in animal and human medicine, which change over time. Collection of data for retail meat isolates was added in 2002. Annual reports of the NARMS surveillance are accessible at the web sites of the CDC, FDA, and USDA (HHS/FDA/CVM 2003, 2004; NARMS 2003a, b, 2004). Data are available from CDC for antimicrobial susceptibility or resistance among zoonotic bacteria associated with human clinical cases (see Table 6a), from FDA for susceptibility among bacteria from retail meats (see Table 6b), and from USDA for susceptibility

[13] microarrays: a medium comprising an automated process for simultaneously matching, on the basis of base pairing rules, thousands of unknown and known DNA samples of $< 200\ \mu$ diameter

Table 6a—Percent resistance among zoonotic bacteria isolated from human clinical cases—United States (Source: NARMS 2003b)

		Year[a]							
Microorganism	**Resistance**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**
Salmonella, all non-Typhi serotypes	None of 14 agents	66						79	78
	2 or more agents	28						16	18
	5 or more agents	12	14				12	9	11
	Ciprofloxacin[b]	0	0	0.1	0.1	0.4	0.2	0.05	0.2
	Nalidixic acid	0.4					3	2	2
	Ceftriaxone	0			0.4			0.2	0.4
	Ceftiofur	4	3	1	2	3	4	4	4
	Ampicillin	21						13	14
	Tetracycline	24						15	16
	Trimethoprim-sulfamethoxazole	4						1	2
Salmonella Typhimurium	None of 14agents	36						60	55
	2 or more agents	58						36	41
	5 or more agents	41	47					27	30
	Salmonella Typhimurium R-type ACSSuT (% of non-Typhi *Salmonella* isolates)	8	9	8				4	6
	Salmonella Typhimurium R-type ACSSuT (% of *Salmonella* Typhimurium isolates)	34	35					21	26
	Ciprofloxacin[b]	0					0.3	0	0
	Nalidixic acid	0.3			0	1	1	1	1
	Ceftriaxone	0						0.3	0.2
	Ceftiofur	4	5	2	2	4	3	4	5
	Ampicillin	50	51					34	36
	Tetracycline	49	53					32	38
	Trimethoprim-sulfamethoxazole	4		4		4	2	2	4
Salmonella Newport	None of 14 agents	82	88	95			65	73	74
	2 or more agents	8	6	3			31	25	25
	5 or more agents	6	4	3		23	27	23	22
	MDR-AmpC resistance pattern (% of non-Typhi *Salmonella* isolates)	0	0					3	2
	MDR-AmpC resistance pattern (% of *Salmonella* Newport isolates)	0	0			22	25	22	21
	Ciprofloxacin[b]	0						0	0
	Nalidixic acid	0	0	0	1	1	0	1	0.5
	Ceftriaxone	0	0	0	0	0	0	1	2
	Ceftiofur	4	4	1		22	27	22	22
	Ampicillin	6	6	3		23	29	24	22
	Tetracycline	8	4	3			30	25	24
	Trimethoprim-sulfamethoxazole	4	4	1	2	4	2	4	1
Campylobacter	None of 6 agents		44			52			51
	2 or more agents		18	18	20	16	21	21	18
	Tetracycline		47			38			38
	Nalidixic acid		20	18	21	16	21	21	19
	Ciprofloxacin		13	13	18		20	20	18
	Erythromycin		3		3				1
Escherichia coli O157	none of 14	85		93		90	91	93	90
	2 or more agents	5	6	5	4	7	5	4	5
	Tetracycline	5	3		3	7	5	3	6
	Ampicillin	1	0		1	3		1	3
	Nalidixic acid	0	0	0	1	1	1	1	1
	Trimethoprim-sulfamethoxazole	0	0	1	1	1	1	1	1

[a] Information is shown for those intermediate years with data points that are outside the range (above or below) of the first and last years.
[b] The breakpoint used by NARMS to classify resistance against ciprofloxacin is MIC $\geq$ 4.0 μg/ml. Decreased susceptibility of *Salmonella* were those with an MIC $\geq$ 0.25 μg/ml.

Table 6b—Percent resistance among bacteria isolated from retail meats—United States (Source: HHS/FDA/CVM 2002; HHS/FDA/CVM 2003)

Microorganism	Resistance	Year[a] 2002	2003
Salmonella, all non-Typhi serotypes (n = 153 isolates in 2002, 212 in 2003)	None of 16 agents	44	40
	2 or more agents	42	51
	5 or more agents	20	26
	8 or more agents	8	6
	Ciprofloxacin[b]	0	0
	Ciprofloxacin decreased susceptibility	3	3
	Nalidixic acid	4	3
	Ceftriaxone	0	0
	Ceftiofur	10	14
	Ampicillin	18	32
	Tetracycline	46	36
	Trimethoprim-sulfamethoxazole	2	0
Salmonella Typhimurium (n = 15 isolates in 2002, 26 in 2003)	Ciprofloxacin[b]	0	0
	Ciprofloxacin decreased susceptibility	0	0
	Nalidixic acid	0	0
	Ceftriaxone	0	62
	Ceftiofur	20	73
	Ampicillin	27	35
	Tetracycline	40	0
	Trimethoprim-sulfamethoxazole		0
Salmonella Newport (n = 8 isolate in 2002, 4 in 2003)	Ciprofloxacin[b]	0	0
	Nalidixic acid	0	0
	Ceftriaxone	0	0
	Ceftiofur	62	50
	Ampicillin	62	50
	Tetracycline	62	50
	Trimethoprim-sulfamethoxazole	0	0
Campylobacter (n = 288 isolates in 2002, 479 in 2003)	None of 5 agents	60	60
	2 or more agents	7	6
	Doxycycline	28	30
	Ciprofloxacin	14	14
	Erythromycin	6	3
Escherichia coli (n = 1065 in 2002, 1258 in 2003)	None of 16	36	36
	2 or more agents	46	48
	Tetracycline	52	48
	Ampicillin	19	21
	Nalidixic acid	2	5
	Trimethoprim-sulfamethoxazole	2	5

among zoonotic bacteria from animals and animal products (see Table 6c). Animal and human isolates currently monitored include nontyphoid *Salmonella, Campylobacter, E. coli*, and *Enterococcus*. The CDC also monitors *Salmonella* Typhi and *Shigella* human isolates.

Prevalence of antimicrobial resistance observed in U.S. monitoring systems. Comparison of human clinical isolates from the early surveillance years with those for 2003 shows a decreasing trend in resistance in many cases but an increasing trend in others (Table 6a). For example, in 2003, 78% of the *Salmonella* isolates (all non-Typhi serotypes) were pansusceptible (susceptible to all 14 antimicrobial agents tested in both 1996 and 2003) compared with 66% in 1996. The percentage of the non-Typhi *Salmonella* isolates that were resistant to two or more antimicrobials decreased from 28% in 1996 to 18% in 2003, and the percentage of non-Typhi *Salmonella* isolates resistant to five or more antimicrobials decreased from 12% to 11%. The most common *Salmonella* serotype tested by NARMS is *Salmonella* Typhimurium. Consequently, the resistance prevalence changes in *Salmonella* Typhimurium greatly influenced and mirrored the prevalences of all *Salmonella* combined. Pansusceptibility of *Salmonella* Typhimurium changed from 38% in 1996 to 55% in 2003. Resistance to two or more antimicrobials decreased from 57% in 1996 to 41% in 2003 and resistance to five or more antimicrobials decreased from 41% to 30%. The second most common *Salmonella* serotype is *Salmonella* Enteritidis. Resistance prevalence is comparatively low for *Salmonella* Enteritidis; 91% of the isolates in 2003 were pansusceptible compared to 74% in 1996. However, in the third most common *Salmonella* serotype, *Salmonella* Newport, the prevalence of resistance increased. In 1996, 86% of the *Salmonella* Newport isolates were pansusceptible; this decreased to 74% in 2003. Resistance to five or more

Table 6c—Percent resistance in zoonotic bacteria isolated from animals and animal products—United States (Source: USDA/ARS 2006)

Microorganism	Resistance	Year[a]						
		1997	1998	1999	2000	2001	2002	2003
Salmonella, all non-Typhi serotypes from all animal sources (diagnostic, slaughter, healthy)	None of 14 agents	66				48		49
	2 or more agents	25				44		43
	5 or more agents	11						25
	8 or more agents	2						14
	Ciprofloxacin[b]	0	0	0	0	0	0	0
	Nalidixic acid	1	1	1	2	2	1	1
	Ceftriaxone	0	1	0	0	0	0	0
	Ceftiofur	1						19
	Ampicillin	12						30
	Tetracycline	27				44		42
	Trimethoprim-sulfamethoxazole	2		3	6			5
Salmonella, all non-Typhi serotypes from cattle slaughter isolates	Ciprofloxacin[b]	0	0	0	0	0	0	0
	Nalidixic acid	0	0	0	0	0	0	0
	Ceftriaxone	0	1	0	0	0	0	0
	Ceftiofur	0						21
	Ampicillin	19	9	12	19	18		28
	Tetracycline	31	24	21	26	26		36
	Trimethoprim-sulfamethoxazole	4	2	2	2	3	2	3
Salmonella, all non-Typhi serotypes from chicken slaughter isolates	Ciprofloxacin[b]	0	0	0	0	0	0	0
	Nalidixic acid	0	0	0	0	0	1	0
	Ceftriaxone	0	0	0	0	0	0	0
	Ceftiofur	0					10	10
	Ampicillin	12		12		9	14	14
	Tetracycline	21	20					27
	Trimethoprim-sulfamethoxazole	0	1	1	0	0	1	0
Salmonella, all non-Typhi serotypes from swine slaughter isolates	Ciprofloxacin[b]	0	0	0	0	0	0	0
	Nalidixic acid	0	0	0	0	0	0	0
	Ceftriaxone	0	0	0	0	0	0	0
	Ceftiofur	1	0		1			4
	Ampicillin	17	13	11	19	12		13
	Tetracycline	51			54	53	58	43
	Trimethoprim-sulfamethoxazole	2	0	1	1	0	2	2
Salmonella, all non-Typhi serotypes from turkey slaughter isolates	Ciprofloxacin[b]	0	0	0	0	0	0	0
	Nalidixic acid	5	2	5	5	5	5	4
	Ceftriaxone	2	0		0	0	0	0
	Ceftiofur	6	0					2
	Ampicillin	13	10			20		19
	Tetracycline	55	46	53		55	54	59
	Trimethoprim-sulfamethoxazole	4	2	4	2	2	2	2
Salmonella Typhimurium from all animal sources (diagnostic, slaughter, healthy)	*Salmonella* Typhimurium R-type ACSSuT (% of non-Typhi *Salmonella* isolates)			6	6		4	4
	Salmonella Typhimurium R-type ACSSuT (% of *Salmonella* Typhimurium isolates)			35				25
	Ciprofloxacin[b]	0		0	0	0	0	0
	Nalidixic acid	2		2	1	5	4	3
	Ceftriaxone	1		1	0	1	0	0
	Ceftiofur	2						27
	Ampicillin	61		63	69		62	56
	Tetracycline	64		64	68			46
	Trimethoprim-sulfamethoxazole	4		9	13	6	6	5
Salmonella Newport from all animal sources (diagnostic, slaughter, healthy)	Ciprofloxacin[b]				0	0	0	0
	Nalidixic acid				0	1	1	0
	Ceftriaxone			1	0	1	1	
	Ceftiofur				75	69	78	74
	Ampicillin				76	72	80	74
	Tetracycline				78	75	83	77
	Trimethoprim-sulfamethoxazole				19			2

(*Continued on next page*)

Table 6c—Continued

Microorganism	Resistance	Year[a] 1997	1998	1999	2000	2001	2002	2003
Campylobacter (slaughter isolates)	Tetracycline		60			45	46	49
	Nalidixic acid		20	13	13	21[c]	21	18
	Ciprofloxacin		13	11	11	20[c]	18	17
	Erythromycin		10	4	9	2	7	9
Escherichia coli (all sources)	Tetracycline				80		36	40
	Ampicillin				21		14	17
	Nalidixic acid				1	8		6
	Trimethoprim-sulfamethoxazole				5	12		10

[a]Information is shown for those intermediate years with data points that are outside the range (above or below) of the first and last years.
[b]The breakpoint used by NARMS to classify resistance against ciprofloxacin is MIC = 4.0 μg/ml. Decreased susceptibility of *Salmonella* were those with an MIC = 0.25μg/ml.
[c]Methods of testing changed in 2001. See NARMS report for more details.

antimicrobials increased from 6% in 1996 to 22% in 2003. In 2003, 21% of the *Salmonella* Newport isolates exhibited at least MDR-AmpC resistance compared with none in 1996. Among *E. coli* O157 human isolates, 89% were pansusceptible in 2003 compared with 85% in 1996, and the percentage of *E. coli* O157 isolates resistant to two or more antimicrobials remained at 5% during this time period. Among *Campylobacter* isolates, 51% were pansusceptible in 2003 compared with 44% in 1997, and 22% were resistant to two or more antimicrobials in 1997 compared with 18% of isolates in 2003.

CDC's annual report for 2003 (NARMS 2003a) provides trends in resistance to clinically important antimicrobials (fluoroquinolones and third generation cephalosporins, for example). The key findings reported are: (1) 18% of *Campylobacter* isolates in 2003 were resistant to ciprofloxacin, compared with 13% in 1997; (2) 2% of non-Typhi *Salmonella* isolates in 2003 were resistant to the quinolone nalidixic acid, compared with 0.4% in 1996; (3) 4% of non-Typhi *Salmonella* isolates in 2003 were resistant to the third generation cephalosporin ceftiofur (an animal drug), compared with 0.2% in 1996. Resistance to the human third generation cephalosporin, ceftriaxone, increased from none in 1996 to 0.4% in 2003.

NARMS data from animal isolates are more complicated to interpret because of the large variety of animal species and sources from which isolates are obtained. Animal isolates originate from federally inspected slaughter and processing facilities, animal health monitoring studies on farms, and veterinary diagnostic laboratories, and are tested for antimicrobial drug susceptibility at the USDA Agricultural Research Service Antimicrobial Resistance Research Unit.

Accurate comparison of trends in resistance among animal isolates requires comparisons within animal species and within the same isolate source (for example, meat, healthy animals, or diagnostic specimens). Further, some comparisons are affected by methodological changes, such as the change in 2001 in methodology for *Campylobacter* that caused an apparent increase in resistance to ciprofloxacin. Unfortunately, the reports do not provide summary information, such as the prevalence of isolates from cattle, swine, or poultry slaughter and processing specimens that are resistant to two or more antimicrobials. Available summary information combines all species of animals, all sources, and all species of *Salmonella*. The summary information for these animal isolates shows increases in resistance to two or more, five or more, and eight or more antimicrobials. These results differ from those of the human *Salmonella* isolates. Resistance to clinically important antimicrobials among animal isolates was unchanged for ciprofloxacin and ceftriaxone (0% each), a third generation cephalosporin used in humans and particularly in children as an alternative to fluoroquinolones (NARMS 2003b). Paradoxically, resistance to ceftiofur (a third generation cephalosporin used in animals) increased from 1% in 1997 to 19% in 2003. At least in this situation, development of resistance to one member of a class of antibiotics does not confer resistance to other members of that class.

In the animal arm of NARMS, the primary or exclusive source of *Campylobacter* isolates is chicken specimens collected at slaughter, in which resistance to tetracycline, erythromycin, and nalidixic acid decreased between 1998 and 2002 (60% to 49%, 10% to 9%, and 20% to 18%, respectively) but increased for ciprofloxacin (13% to 17%). In the retail arm, in 2002 and 2003, 14% of the *Campylobacter* isolates were resistant to ciprofloxacin. In 2002, 6% were resistant to erythromycin and in 2003, 3% were resistant to the antibiotic. Instead of testing for resistance to tetracycline, the retail arm tested for resistance to doxycycline, which was 28% in 2002 and 30% in 2003. The resistance trends between 1997 and 2002 for *Campylobacter* isolates from humans were the same as for isolates from animals between 1998 and 2002 for tetracycline (decrease), nalidixic acid (increase), and ciprofloxacin (increase), but were opposite the trends for erythromycin (increase in humans; decrease in animals and retail meats).

Except for *Salmonella*, there is a paucity of data on prevalence of antimicrobial susceptibility phenotypes among foodborne pathogens associated with foods imported into the United States. Zhao and others (2003a) evaluated the susceptibility to 17 antimicrobials of 187 *Salmonella* isolates representing 82 serotypes recovered by FDA field laboratories from 4072 foods imported into the United States in the year 2000. They found that 8% of the isolates were resistant to at least one antimicrobial and 2.7% were resistant to three or more. Of the isolates that were resistant to at least one antibiotic, 12 isolates were recovered from seafood and the remaining three were recovered from fresh produce or cheese; 10 of them were isolated from food imported from Asia and the other five were recovered in foods from Mexico, Ecuador, Canada, or Denmark. One *Salmonella* Derby isolate from frozen anchovies imported from Cambodia was resistant to six antimicrobials, including ampicillin, amoxicillin/clavulanic acid, and chloramphenicol. Nine isolates exhibited resistance to tetracycline, seven to sulfonamides, five to streptomycin, and four (from catfish or tilapia from Taiwan or Thailand) demonstrated resistance to nalidixic acid.

Kiessling and others (2002) tested susceptibility to 11 antimicrobial agents of 502 isolates recovered from domestic and imported food and related products by FDA between Oct. 1, 1999

and Sept. 30, 2000; 92 of the cultures were isolated from domestic samples and the remainder were isolated from imported products. Of the 247 isolates showing resistance or intermediate resistance, 23% were from U.S. products and 74% were from imported items. Marked differences were observed in the proportions of resistant isolates from different product types. Many of the resistant isolates originated from domestic and imported pig ear dog treats; those from human food sources originated from seafood, and, to some extent, vegetables. Resistance to seven antimicrobials was observed in isolates from frozen eel imported from Vietnam and frozen anchovies from Cambodia; resistance to six antimicrobials was seen in isolates from pig ears from Canada and frozen tilapia from Taiwan; resistance to five antimicrobials was seen in basil isolates from Egypt, romaine lettuce from Illinois, poultry meal from Tennessee, three pig ear samples from Canada, and two pig ear samples from California; resistance to four antimicrobials was observed in isolates from frozen catfish from Thailand, herbs from France, and pig ears from Venezuela, North Carolina, Spain, and California. Remarking on the distribution of MDR-phenotypes among different *Salmonella* serotypes, the authors noted that *Salmonella* Derby showed the highest frequency (70%) of multi-resistance, followed by *Salmonella* Typhimurium (>50%), whereas none of the *Salmonella* Newport, *Salmonella* Muenchen, or *Salmonella* Thompson isolates was resistant to two or more antimicrobials, and only one *Salmonella* Enteritidis isolate was multiresistant.

Analyzing isolates collected from seafood products between 1999 and 2003, Kiessling and others (2004) found that 25% of *Salmonella* isolates from Thailand were resistant to two or more antibiotics, as were 23% of *Salmonella* isolates from Bangladesh, and 21% of those from the Honduras.

Canada

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) is a recently developed surveillance system that is similar to the U.S. NARMS. Unlike the U.S. NARMS, however, CIPARS provides a consolidated report of passive surveillance data on *Salmonella* from human clinical cases, active surveillance data collected from slaughterhouses, and passive surveillance data on *Salmonella* from animal clinical specimens (Health Canada 2004).

Europe

The European Antimicrobial Resistance Surveillance System (EARSS) is an international network of national surveillance systems. EARSS performs on-going surveillance of antimicrobial susceptibility in *Streptococcus pneumoniae, S. aureus, E. coli*, and *E. faecalis/faecium* causing invasive infections in humans, and monitors variations of antimicrobial resistance over time and from place to place. By the first quarter of 2003, about 700 microbiological laboratories serving some 1100 hospitals from 28 countries had provided susceptibility data on about 175000 invasive isolates (EARSS 2004). Another Europe-based surveillance network of interest is Enter-Net (2003), an international surveillance network for human gastrointestinal infections of *Salmonella* and verocytotoxin-producing *E. coli* and antimicrobial resistance. In addition, Denmark and Norway have independent surveillance systems.

Denmark

Another long-standing surveillance system is the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP 2004), which was established in 1995 as a coordinated national surveillance and research program for antimicrobial consumption and resistance in bacteria from animals, food, and humans. DANMAP is unique in that it provides temporal relationships between antimicrobial usage and resistance, although CIPARS is beginning to collect on-farm usage data.

Norway

Reports similar to DANMAP are available from Norway (NORM/NORM-VET 2002) and Sweden (SVARM 2002). The Norwegian surveillance program for antimicrobial resistance in human pathogens was established in 1999. The NORM-VET monitoring program for antimicrobial resistance in the veterinary and food production sectors was established in 2000.

Investigations of Resistance among Specific Genera

Salmonella

Lee and others (1994) compared the proportion of resistant *Salmonella* isolates from human patients in selected U.S. counties during 1979 to 1980 and 1989 to 1990. The percentage of isolates that were resistant to ≥1 of 12 antimicrobial agents was 17% in 1979 to 1980, 26% in 1984 to 1985, and 31% in 1989 to 1990; the percentage of infections by MDR-strains was 12% in 1979 to 1980, 17% in 1984 to 1985, and 25% in 1989 to 1990. Of the human isolates addressed in NARMS, resistance to ≥1 of 14 antimicrobials was 34% in 1996 and 22% in 2003; resistance to ≥2 of 14 antimicrobial agents was 28% in 1996 and 18% in 2003. These comparisons indicate a peak in 1996 and a subsequent decline back to 1983 to 1985 levels. Similarly, Threlfall and others (2004) reported that the peak year for MDR-*Salmonella* Typhimurium DT104 human infections in England and Wales was also 1996.

One of the most recognized *Salmonella* serotypes in both animal and human illnesses is *Salmonella* Typhimurium. Of particular concern is the increasing number of MDR-resistant *Salmonella* Typhimurium isolates, including definitive phage (virus specific to a bacterium) type 104 (DT104). This *Salmonella* strain is usually resistant to at least five antimicrobial agents—ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT). Antimicrobial resistance among *Salmonella* isolates appears to be increasing on a global scale, although a large part of this rise may be attributed to the clonal spread of MDR-varieties, including *Salmonella enterica* Typhimurium DT104 (Besser and others 2000; Glynn and others 1998; Ribot and others 2002).

Salmonella Typhimurium DT104 was first associated with seagulls, and then cattle and humans in England, although it most likely did not originate there. *Salmonella* Typhimurium DT104 has caused serious illnesses in many animal species including food animals (Davis and others 1999; Evans and Davies 1996), companion animals (CDC 2001a; Hudson 2000; Wall and others 1996), and wildlife (Foreyt and others 2001; Helm 1999). Many food animal species, although asymptomatic, can serve as reservoirs or carriers for *Salmonella* Typhimurium DT104 (Abouzeed and others 2000; Baggesen and Aarestrup 1998; Benson and others 1997; Imberechts and others 1998; Rajashekara and others 2000). Several instances of transmission of *Salmonella* Typhimurium DT104 from infected animals to humans have been reported (CDC 2001b; Spake 1997). In addition, several human outbreaks associated with DT104 have been linked to consumption of dairy products (Cody 1999; Villar and others 1999) and beef (Evans and Davies 1996).

The prevalence in the United States of ACSSuT-resistant *Salmonella* Typhimurium increased from 0.6% during 1979 to 1980 to 34% in 1996 (Glynn and others 1998). Among the

Salmonella Typhimurium isolates from humans submitted to NARMS, the prevalence of isolates at least ACSSuT-resistant in 2003 was 26% compared with 34% in 1996 (NARMS 2003b). This penta-resistance pattern in *Salmonella* Typhimurium is often indicative of phage type DT104, but on rare occasions other antibiotic resistance patterns have been identified in DT104 as well (Abouzeed and others 2000; Imberechts and others 1998; Low and others 1997; Rajashekara and others 2000). Some DT104 isolates have acquired resistance to trimethoprim and aminoglycosides as well as to quinolones (Low and others 1997; Molbak and others 1999; Threlfall and others 1996).

Most resistant DT104 isolates have a unique MDR-chromosomal gene cluster encoding the complete spectrum of the ACSSuT phenotype (Arcangioli and others 1999; Briggs and Fratamico 1999; Ridley and Threlfall 1998; Threlfall and others 1996). This gene cluster typically consists of a 12.5 kb chromosomal locus with flanking integrons (Arcangioli and others 1999; Briggs and Fratamico 1999). *Salmonella* Typhimurium DT104 is also resistant to chloramphenicol and the veterinary analog florfenicol (Bolton and others 1999).

Chloramphenicol/florfenicol resistance is due to *flo*, a putative drug efflux pump first described in the fish pathogen *P. dansalae* (Kim and Aoki 1996). This phenicol resistance gene has been found in other *Salmonella*, *E. coli,* and *Klebsiella pneumoniae* (Cloeckaert and others 2000a, 2000b 2001; Keyes and others 2000; Sanchez and others 2002). Unlike DT104, *flo* resides on plasmids in most *E. coli* isolates (Cloeckaert 2000a; Keyes and others 2000; Sanchez 2002), and AmpC plasmids of *Salmonella* Typhimurium and *Salmonella* Newport (Doublet and others 2004). In *Salmonella* Typhimurium DT104, the *flo* resistance gene occurs next to that for tetracycline resistance (efflux pump *tetG)*. Both genes are further flanked in the chromosome by class 1 integrons (Boyd and others 2001; Briggs and Fratamico 1999). These integrons in DT104 encode for resistance to streptomycin, sulfonamides, and ampicillin (Briggs and Fratamico 1999).

Arrangement of these drug resistance genes within the bacterial chromosome was once considered unique to DT104, but an MDR-locus has been identified in *Salmonella enterica* Agona (Boyd and others 2001; Cloeckaert and others 2000b), *Salmonella* Paratyphi B, and *Salmonella* Albany (Doublet and others 2003; Meunier and others 2002), suggesting that the MDR-gene locus is transferable between serotypes. It has been shown experimentally that the DT104 MDR-cluster can be efficiently transduced[14] by P22-like phages (Schmieger and Schicklmaier 1999). In addition, the occurrence of a gene encoding a putative resolvase enzyme that demonstrates greater than 50% identity with the Tn*3* resolvase family (Arcangioli and others 1999) upstream of the first class 1 integron in the MDR-locus suggests that the MDR-gene cluster could be part of a much larger transposon or pathogenicity island.

More recently, another MDR-*Salmonella*, Newport-MDR-AmpC, has been undergoing epidemic spread throughout the United States in both animals and humans (CDC 2003; Dunne and others 2000). In addition to the penta-resistance phenotype usually observed in *Salmonella* Typhimurium DT104, Newport-MDR-AmpC exhibits resistance to amoxicillin/clavulanic acid, cephalothin, cefoxitin, and ceftiofur, and decreased susceptibility to ceftriaxone (MIC $>$ 16 μg/mL). Some *Salmonella* Newport MDR-AmpC strains also show resistance to gentamicin, kanamycin, and trimethoprim/sulfamethoxazole. The prevalence of Newport-MDR-AmpC among *Salmonella* Newport isolates from humans in the United States increased from 0% during 1996 to 1997 to 21% in 2003 (NARMS 2003b). In 2003, 2% of the non-Typhi *Salmonella* isolates were *Salmonella* Newport MDR-AmpC, compared with none in 1996. At least 26 states have isolated MDR-*Salmonella* from humans, cattle, or ground beef. Raw or undercooked ground beef was implicated as the vehicle of a multistate outbreak of *Salmonella* Newport MDR-AmpC (Anonymous 2002). A retrospective case-control study showed that infection with MDR-*Salmonella* Newport (MDR-AmpC) was domestically acquired and associated with dairy farm exposure. Furthermore, *Salmonella* Newport isolates recovered from both humans and cattle had either indistinguishable or closely related antimicrobial susceptibility profiles and DNA fingerprints (Gupta and others 2003). A recent study by Berge and others (2004) that analyzed human, animal, and environmental MDR-*Salmonella* Newport isolates recovered during 1988 to 2001 indicated that several of the isolates collected since 1998 appeared to be from a clonal population that included human, environmental, and bovine sources in a wide geographic region. An epidemiologic investigation in Canada in 2002 determined human infections with *Salmonella* Newport phage type 14 strains resistant to ceftazidime and cefoxitin were associated with handling pet treats containing dried beef (Pitout and others 2003).

Zhao and others (2003b) showed that among 87 human and food animal *Salmonella* Newport isolates, 60% were identified as Newport MDR-AmpC, of which 53% were from humans, 93% from cattle, 70% from swine, and 30% from chickens. All 53 *Salmonella* Newport MDR-AmpC isolates possessed a cephalomycinase, encoded by the bla_{CMY} gene. This extended-spectrum β-lactamase has been associated with resistance to narrow-, expanded-, and broad-spectrum cephalosporins, and is widespread in many other Gram-negative enteric pathogens as well.

Isolates from culture collections and directly associated with outbreaks were evaluated retrospectively for antimicrobial resistance. β-lactam and cephalosporin resistance in *Salmonella* has been attributed to several distinct classes of β-lactamase/cephalosporinases (Bauernfeind and others 1996; Hanson and others 2002; Makanera and others 2003); the recent ceftriaxone-resistant, clavulonic acid resistant phenotype identified in *Salmonella,* however, is associated with plasmid-borne AmpC CMY-2 (Chen and others 2004; Koeck and others 1997; Navarro and others 2001; Zhao and others 2001b). The AmpC CMY-2 gene appears to have originated in *Citrobacter freundii* (Dunne and others 2000) and it has since been disseminated worldwide to *Salmonella* and other *Enterobacteriaceae* (Navarro and others 2001; Odeh and others 2002), including those from animal sources (Sanchez and others 2002; Yan and others 2004a; Zhao and others 2001b). Spread of *ampC* CMY-2 and the associated, extended-spectrum, cephalosporin resistance (Winokur and others 2001; Yan and others 2004a) as well as resistance to other drugs (Doublet and others 2004) appears to be attributed to a common plasmid and in part to a class 1 integron and its associated resistance genes residing on the plasmid (Rankin and others 2002; Zhao and others 2003b).

Fluoroquinolone and ceftriaxone-resistant *Salmonella* are of particular concern to public health because fluoroquinolone, ciprofloxacin, and third generation cephalosporins such as ceftriaxone are agents most commonly used for treating invasive *Salmonella* infections in adults and children, respectively (Angulo and others 2000; Fey and others 2000). Thus, the need continues for increased surveillance on a global basis of antimicrobial resistant phenotypes among *Salmonella* spp. of animal and human origin, with specific emphasis on susceptibility to drugs used to treat infection.

[14] transduced: having its genetic constitution changed via genetic recombination through the transfer of DNA from a lysed bacterium via bacteriophage

Campylobacter

Campylobacter is a naturally transformable microorganism (Wang and Taylor 1990) that is capable of acquiring a diverse array of Gram-positive (Werner and others 2001) and Gram-negative (Pinto-Alphandary and others 1990) resistance genes. Since the late 1980s, resistance to fluoroquinolones has been increasing among *Campylobacter* isolates, especially in Europe, while the level of erythromycin resistance has not changed (Piddock 1995). Gaudreau and Gilbert (1998) compared resistance levels over time among *C. jejuni* human isolates, and found that none of the strains from any of the three time periods (1985 to 1986, 1992 to 1993, and 1995 to 1997) was resistant to erythromycin; and, although there was no significant increase in resistance to nalidixic acid or ciprofloxacin from 1985 to 1986 and 1992 to 1993, there was a significant increase between 1992 to 1993 and 1995 to 1997. Lucey and others (2002) compared resistance of *C. jejuni/coli* in Ireland. Between the periods of 1996 to 1998 and 2000, the erythromycin resistance levels remained low (2%) among human isolates, but ciprofloxacin resistance increased from 0% to 30%. Nachamkin and others (2002) reported an increase of fluoroquinolone resistance (from 21% in 1995 to 40% in 2001) among human *C. jejuni* isolates in Pennsylvania, and level erythromycin resistance (remaining less than 5%). Smith and others (1999) reported that the proportion of nalidixic acid resistance among human *C. jejuni* isolates from Minnesota increased from 1% in 1992 to 10% in 1998. The authors noted that infection was associated primarily with foreign travel and fluoroquinolone use, although the number of quinolone-resistant infections acquired domestically also increased between 1996 and 1998. Summarizing the antimicrobial resistance surveillance data for human isolates from a sentinel county health study during 1989 to 1990 and NARMS during 1997 to 2001, Gupta and others (2004) reported that during 1989 to 1990 none of the isolates was ciprofloxacin-resistant and 1% was resistant to nalidixic acid, but later ciprofloxacin resistance prevalence was 12% in 1997, 14% in 1998, 18% in 1999, 14% in 2000, and 18% in 2001, and erythromycin resistance prevalence was 1% in 1997 and 2% in 2001.

Antibiotic resistance in *Campylobacter* develops at the genetic level, through the acquisition of point mutations in genes encoding DNA gyrase (gyrA [Ge and others 2003; Wang and others 1993]), 23S rRNA (Ge and others 2003; Niwa and others 2001), activation of resident MDR-efflux pumps (Lin and others 2002), or acquisition of foreign genes that either alter the antibiotic (Pinto-Alphandary and others 1990; Werner and others 2001) or its target (LeBlanc and others 1988). Resistance to erythromycin is attributed to reduced binding to the ribosome (Yan and Taylor 1991). Although an MDR-efflux pump has been identified in *Campylobacter*, it does not appear to be responsible for the high MIC levels associated with erythromycin (Chatzipanagiotou and others 2002). Unlike *Salmonella*, other *Enterobacteriaceae* and pseudomonads, *Campylobacter* is naturally susceptible to macrolide antibiotics.

E. coli

The *E. coli* O157:H7 strains initially associated with human illness were susceptible to most antimicrobials used against Gram-negative pathogens, but during the past two decades the antimicrobial resistance profile of *E. coli* O157:H7 has increased. Early studies showed that approximately 3% (5 of 174) of *E. coli* O157:H7 strains were resistant to antibiotics (Ratnam and others 1988). Similarly, only 2 of 200 *E. coli* O157:H7 strains collected by CDC between 1983 and 1985 were resistant to antibiotics (Bopp and others 1987). Screening of 125 *E. coli* O157: H7 (n = 118) and *E. coli* O157: NM (n = 7) isolates, the majority of which were collected during the early 1990s, revealed that 24% were resistant to at least 1 antimicrobial and 19% were resistant to 3 or more. The significance of the resistance is debatable, however, because antibiotic treatment of illness caused by *E. coli* O157:H7 is generally contraindicated.

The antimicrobial resistant profiles reported for *E. coli* O157:H7 appear fairly consistent between studies. Among *E. coli* O157:H7 isolates of bovine and human origin (n = 663 and n = 238, respectively) collected between 1997 and 2000, 7% of bovine and 12% of human isolates were resistant to one or more antimicrobials (Wilkerson and others 2004). As in previous studies, tetracycline resistance was the most common, followed by streptomycin resistance. Resistance profiles of enterohemorrhagic *E. coli* (EHEC) in the United States are similar to those reported in other countries. A recent report indicated that EHEC were susceptible to quinolones and gentamicin, but some isolates were resistant to tetracycline and cephalothin (Klein and Bulte 2003). Compared with other foodborne pathogens or other *E. coli* isolates, the level of antimicrobial resistance of *E. coli* O157:H7 is generally low and limited to tetracycline, streptomycin, and sulfamethoxazole.

Shigella

Shigella accounts for only a small fraction of the total cases of foodborne illnesses occurring in the United States (Mead and others 1999; Shiferaw and others 2004). The pathogen is generally transmitted person-to-person by the fecal-oral route, but can also be spread indirectly by fecal contamination of food or water. Many reported outbreaks of shigellosis are linked to contamination of product by food handlers and are often attributed to poor food handler hygiene (Lew and others 1991; Rooney and others 2004). Contamination of crops with *Shigella* may occur through application of contaminated human waste to fields or contaminated irrigation water. Equally probable, crops could be contaminated during harvest by farm workers shedding the pathogen. Isolates are often resistant to multiple antimicrobials. Because *Shigella* requires a human host; resistance in the microorganism is due to human rather than agricultural antibiotic use. Shigellosis is more common in developing countries, and, therefore of greater concern than in developed countries. With a global marketplace, however, food production and handling practices in one country can precipitate foodborne illness in other countries.

The outbreak strain of a large shigellosis outbreak in 1987, likely resulting from transmission via food and water and person-to-person spread, was resistant to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole (TMP-SMZ [Wharton and others 1990]). Epidemiologic evidence suggests that an outbreak that occurred in Norway in 1994 was associated with contaminated iceberg lettuce imported from Spain (Kapperud and others 1995), although presence of *Shigella* in the food was not documented retrospectively. Of 11 isolates of *S. sonnei* from patient stool samples, 10 were susceptible to the 13 antimicrobials tested and 1 was resistant to ampicillin.

S. sonnei isolates from a nationwide outbreak in 2000 involving 406 people and traced to commercially prepared 5-layer dip consisting of beans, salsa, guacamole, nacho cheese, and sour cream were resistant to ampicillin and TMP-SMZ. In the United States, TMP-SMZ resistance is worrisome because this is the treatment combination of choice for shigellosis. Fortunately, the isolate was susceptible to fluoroquinolones.

The potential for the spread of antimicrobial resistant *Shigella* from other countries to the United States should not be ignored. Tauxe and others (1990) evaluated *Shigella* isolates for resistance to 12 antimicrobial agents, and reported that 32% of isolates were resistant to ampicillin and 7% were resistant to TMP-SMZ. Among

isolates associated with foreign travelers, 20% were TMP-SMZ-resistant while only 4% of isolates from those without foreign travel history were TMP-SMZ-resistant. The percentage of *Shigella* resistant to ampicillin and TMP-SMZ is increasing in the United States and is now approaching that seen in developing countries where antimicrobial usage is often unrestricted (Bhattacharya and others 2003). A recent study from south Asia indicates that all *Shigella* isolates evaluated were resistant to ampicillin, tetracycline, nalidixic acid, and ciprofloxacin (Bhattacharya and others 2003). Perhaps most alarming is that small outbreaks of shigellosis due to ciprofloxacin-resistant strains have been reported, underscoring the potential role that food handlers and agricultural workers in foreign countries may have on occurrence of MDR-*Shigella* in the United States. Of note, however, changes in agricultural antibiotic use will have no effect on resistance in *Shigella*.

L. monocytogenes

Few studies have examined the prevalence of antibiotic resistance in *L. monocytogenes*, a microorganism generally associated with RTE meats, smoked seafood, and dairy products derived from raw milk. Because *L. monocytogenes* is widespread in the environment, most cases of food contamination result from post-processing contamination. In a survey of 84 clinical isolates of *L. monocytogenes* collected in 3 time periods between 1955 and 1997, rates of resistance to penicillin, ampicillin, erythromycin, tetracycline, and chloramphenicol did not increase (Safdar and Armstrong 2003). Resistance to ampicillin and gentamicin, used in the treatment of listeriosis, was observed in 9.2% and 2% of isolates, respectively. Walsh and others (2001b) examined *Listeria* spp. isolates from Irish retail food and found resistance to tetracycline the most frequent (6.7%); among *L. monocytogenes,* prevalence of resistance to one or more antibiotics was 0.6%, whereas 19.5% of *L. innocua* isolates exhibited some form of resistance. These results suggest that the ability to acquire or develop resistance is species specific. There are a few reports of studies that examined the antimicrobial resistance of bacteria isolated from produce. Prazak and others (2002) examined *L. monocytogenes* isolates from cabbage, environmental, and water sources at various cabbage farms and packing sheds in Texas, and found that 95% (20 of 21) were resistant to two or more antimicrobial agents and 85% (17 of 20) were resistant to penicillin. Because penicillin-resistant *L. monocytogenes* have not previously been reported for human, food, or environmental samples, this study points to an increase in the potential threat that this pathogen poses to human health.

Commensals

Antibiotic resistance has been observed in the natural flora of a number of food animal isolates and retail foods. Schlegelova and others (2002) found that 36 of 49 nonpathogenic *E. coli* isolates from 111 bulk milk samples were resistant to one or more antimicrobials. More than half of *E. faecium* isolates from 82 poultry farms were resistant to ciprofloxacin (Hayes and others 2004); 69% of *E. faecalis* isolates were resistant to erythromycin and 71% of were resistant to penicillin. *Pseudomonas fluorescens* resistant to as many as 6 antibiotics were isolated from raw carrots (Hamilton-Miller and Shah 2001) and generic *E. coli* resistant to at least 1 (19%) or 2 (12%) antimicrobial agents were isolated from apple cider (Senkel and others 2003).

Data suggest that new antimicrobial-resistant phenotypes have emerged among foodborne *E. coli*, with resistance to frontline antimicrobials (including TMP-SMZ, third generation cephalosporins, and fluoroquinolones) occurring among *E. coli* isolates recovered from retail meats (Schroeder and others 2002, 2003). Wang and others (2005) detected antibiotic-resistant microorganisms, at levels ranging from 10^2 to 10^7 CFU, in the majority of retail foods examined, including raw foods, such as meat and shrimp, and RTE items, such as cheeses and salads. Wang and others (2005) detected antibiotic resistance-encoding genes in resistant isolates; *Streptococcus thermophilus,* an industrially important LAB, was found to be a major host for Tet and Em resistance genes in cheese microbiota. The authors found an industrially important LAB, *S. thermophilus*, to be a dominant host for both tetracycline and erythromycin resistance genes among cheese microbiota; *L. lactis* and *Leuconostoc* spp. isolates were also found to carry antibiotic resistance genes.

Although the phenotypic expression of resistance, as indicated via MIC values, among commensals has little meaning because the microbes are not clinically relevant, the resistance genotype is important because it enables these microbes to serve as reservoirs of resistance determinants that may be transferred to pathogenic bacteria. It is speculated that horizontal acquisition is responsible for the occurrence in *L. monocytogenes* of a plasmid containing multiple antibiotic resistance genes with high homology to one that is common in enterococci–streptococci (Poyart-Salmeron and others 1990).

Although limited information exists about the transfer of resistant bacteria and genes between companion animals and humans, less is known about the potential for exchange with or among commensal bacteria.

Genotype measurement has the advantage that it is not dependent on the expression of the resistance genes for detection. But currently there are no established standards for measuring resistance on the basis of genotype. There are few data regarding the expression of resistance genes in commensal bacteria and ability to acquire resistance genes but not express them. Such "nonexpressing" bacteria would remain sensitive to the antibiotic while carrying a potentially transmissible resistance gene.

Enterococci and Staphylococci

Much attention is given in the clinical profession to vancomycin resistance in enterococci and methicillin resistance in staphylococci. Since foods could potentially be a source of enterococci (Franz and others 1999) and staphylococci, it is important that these genera be considered in food safety discussions. The food safety concern associated with *S. aureus*, however, is with the microbe's enterotoxin, not the microorganism itself. Thus, although MRSA is a major concern for nosocomial infections, and was implicated in an outbreak in coleslaw, the resistance profile is not of particular concern with respect to food safety (Jones and others 2002). Although it has been suggested that food animals can contribute to transfer of vancomycin-resistant enterococci (VRE) to humans (Bates and others 1994), a large survey of U.S. meat-processing facilities revealed a demonstrated lack of high level vancomycin resistance among enterococcal isolates (Bodnaruk and others 2001). VRE in the United States is associated with hospital-acquired rather than community-acquired infections, as has been suggested to occur in Europe. This difference is attributed primarily to the use of avoparcin in animal agriculture in Europe between 1975 and 1997; the antimicrobial has never been approved for use in the United States.

Resistance in Other Areas of Investigation

Dairy cattle

The impact of antimicrobial use on resistance has been examined for specific types of animals and situations, such as use of antibiotics on dairy farms to prevent and treat mastitis. Makovec and Ruegg (2003), for example, investigated resistance patterns of major mastitis-causing pathogens isolated from dairy cow milk

samples between Jan 1994 and Jun 2001. They found that percentages of resistance among some pathogens increased while percentages of resistance among others decreased during the course of the study. More specifically, the percentages of Gram-positive pathogens resistant to various β-lactam antimicrobials did not increase and some decreased. The percentage of *S. aureus* isolates resistant to penicillin decreased from 49% to 30%, and the percentage of *Streptococcus* isolates resistant to penicillin decreased from 6% to 1%. And, for several pathogens, percentages of isolates resistant to sulfisoxazole and to trimethoprim-sulfamethoxazole decreased. None of the pathogens exhibited a significant increase in the percentage of isolates resistant to novobiocin–penicillin. On the other hand, percentages of *S. aureus*, *E. coli*, *Enterobacter*, *Enterococcus*, and *Pasteurella* isolates resistant to erythromycin increased, percentages of *Staphylococcus* and *S. aureus* isolates resistant to lincomycin increased, and percentages of coagulase-negative *Staphylococcus* isolates resistant to pirlimycin increased. Similar studies conducted between 1994 and 2000 found no indication overall among mastitis isolates of increased resistance to antibiotics commonly used in dairy cattle (Erskine and others 2002). Moreover, a subcommittee of the National Mastitis Council Research Committee, which examined trends in resistance to drugs used to treat bovine mastitis, concluded that scientific evidence does not indicate widespread emergence of resistance among mastitis pathogens (Erskine and others 2004). Although resistance to antibiotic drugs among mastitis pathogens has been well documented for nearly four decades, there is no evidence to suggest that this is either an emerging or progressing phenomenon.

Aquaculture

Antibiotic resistance in bacteria from Mississippi catfish was first reported by Johnson (1991). Upon evaluating *E. ictaluri* isolates from diseased fish, he determined that 1.1% of isolates were resistant to oxytetracycline, 4.2% to sulfadimethoxine/ormetoprim 5:1, and 5.8% to both antibiotics. In addition, 36% of *Aeromonas* spp. isolates were resistant to oxytetracycline and 7.7% were resistant to sulfadimethoxine/ormetoprim 5:1. In a subsequent study by Hawke and Thune (1992), none of 86 strains of *Flavobacterium columnare* was resistant to oxytetracycline, but 3.5% was resistant to sulfadimethoxine/ormetoprim 5:1.

Drug resistance in strains of *E. ictaluri* in catfish in the 1980s and early 1990s resulted from the acquisition of an R-plasmid (Cooper and others 1993). The R-plasmid possessed a high degree of homology to an R-plasmid from a tribrissen-resistant strain of *E. coli* (strain 1898). This *E. coli* carried genes for resistance to tetracycline, streptomycin, trimethoprim, and sulfamethoxazole, and was isolated from a case of equine cystitis. The mechanism of drug resistance in strains of *Aeromonas* and *Flavobacterium* has not yet been determined.

A steady downward trend in antibiotic resistance prevalence has been seen among *E. ictaluri* isolated from Mississippi catfish farms between 1997 and 2003 (NWAC 2004). Resistance to both sulfadimethoxine/ormetoprim 5:1 and oxytetracycline declined from the relatively high level of 5.8% reported by Johnson (1991) to 1.1% by 1999 and 0% by 2002. This decline is believed to be a direct result of changes in farm management strategies and decreased antibiotic use.

Camus (2001) reviewed the literature on antibiotic susceptibility of *Streptococcus iniae* strains isolated from tilapia for which no antimicrobials are approved, and concluded that most isolates from the United States were uniformly susceptible to florfenicol, gentamicin, kanamycin, furazolidone/nitrofurantoin, oxytetracycline, and sarafloxacin, and had intermediate susceptibility to amoxicillin, ampicillin, enrofloxacin, and erythromycin. The isolates were considered innately resistant, however, to sulfadimethoxine/ometoprim 5:1. The rapid acquisition of drug resistance among strains of *Photobacterium damselae subsp. piscicida* was shown to be mediated by an R-plasmid (Hawke and others 2003), which confers resistance to both Romet and Terramycin.

In Japan, a variety of antibiotics has been used during the past 20 years to treat bacterial disease in mariculture. This has led to antibiotic resistance, attributed to R-plasmids, in *P. damselae susp. piscicida* (Aoki and Kitao 1985). Takashima and others (1985) reported R-plasmid-mediated resistance to chloramphenicol, tetracycline, ampicillin, kanamycin, and sulfamonomethoxine in Japanese aquaculture in the early 1980s. More prudent use of antibiotics has reduced this trend in recent years (Aoki 2005).

Plants and produce

Streptomycin resistance is very common among bacteria in orchards sprayed with this antibiotic (McManus and others 2002). Oxytetracycline resistance among target pathogens has not been detected. A study of leaves and blossoms from two apple orchards showed that 0% and 47% of bacteria (mostly Gram-negative) were resistant to tetracycline (Schnabel and Jones 1999). Of note, oxytetracycline had been used in only one orchard, and higher rates of resistance were seen from those isolates. Streptomycin resistance was seen in 26% of bacteria isolated from blossoms and 84% of bacteria isolated from leaves (Schnabel and Jones 1999). Most bacteria resistant to tetracycline were also resistant to streptomycin. There are no studies determining whether human and animal health in these areas is compromised as a result of such use. The limited applications (averaging two to four times a season) and exposure, and required precautions for application as well as re-entering sprayed areas may have contributed to lack of resistance or its recognition in humans. In addition, genetic mechanisms of streptomycin resistance in plants currently appear to be distinct from those reported in pathogens isolated from humans (McManus and others 2002).

Currently, very little data exist to indicate the prevalence of antibiotic or antimycotic-resistant bacteria or fungi associated with raw fruits and vegetables, and available data are not consistent. In reports of fruit and vegetable contamination throughout the various stages of the food system, antimicrobial-resistant bacteria or fungi are not often addressed (Buck and others 2003; FDA 1998). Johnston and Jaykus (2004) reported finding that fresh produce harbors strains of enterococci that are resistant to many commonly used antibiotics, and that prevalence (or degree) of antibiotic resistance was lower than that found in retail meats.

Because the monitoring and surveillance of foodborne human pathogens generally neglect the cross-over pathogens that can cause disease in plants and humans (Tan 2002; Taylor and others 2001), prevalence of the cross-over microbes is not known. Thus, without such data, appropriate dietary recommendations for people, particularly subpopulations at greater risk of microbial infection than the general population, cannot be made.

The most common fungal pathogens associated with plants that are capable of infecting humans are *Aspergillus fumigatus* and *Fusarium* spp. These pathogens are intrinsically resistant to azoles, which are among the ten antimycotic drugs currently approved by the FDA for treating systemic human fungal infections (Hof 2001). Nevertheless, there are conflicting views about the severity and significance of fungal resistance to these agents (EC 2002). Although there are no common antimycotics used in agriculture and human medicine, the prevalence of azole fungicide use is worrisome for the potential for negative impacts on human health.

Case Study: Organic Foods

Organic farming is one of the fastest growing segments of U.S. agriculture; during the past decade, the market for organic food has increased 20 to 25% each year, five times faster than general food sales (Greene 2001). The key principles and practices of organic food production aim to encourage and enhance biological cycles within the farming system to maintain and increase long-term soil fertility, minimize all forms of pollution, avoid the use of synthetic fertilizers and pesticides, maintain genetic diversity of the production system, consider the wider social and ecological impact of food production and processing, and produce food of high quality in sufficient quantity. Additionally, organic livestock production has the goal of sustaining animals in good health and realizing high animal welfare standards (Sundrum 2001).

The question of whether organically produced food poses any greater microbiological risk to consumers than conventionally grown food has not yet been sufficiently addressed (Bourn and Prescott 2002). Organic production practices, such as the use of animal manures and prohibition of some food additives and food processing techniques, may increase the risk of microbiological contamination and foodborne illness. A limited number of studies of organic fresh vegetables indicated no significant difference in the microbiological safety of organic and conventional vegetables (Johannessen and others 2004; Mukherjee and others 2004), and that the use of manure did not affect the bacteriological quality of these products (Johannessen and others 2004). A study in Minnesota, however, found that organic lettuce had greater prevalence (22%) of *E. coli* than conventional lettuce (Mukherjee and others 2004), and 1 in Denmark found that *Campylobacter* contaminated all 22 organic broiler flocks but only a third of 79 conventional flocks (Heuer and others 2001). Mukherjee and others also reported that organic samples from farms using manure or compost aged less than 12 months had a prevalence of *E. coli* 19 times greater than that of farms using older materials.

Organic meat production may involve potentially higher microbiological safety risks simply due to raising of animals in an outdoor environment, use of slow-growing breeds (longer grow-out period), prohibition of antimicrobial use, and use of very small slaughtering facilities (Engvall 2001; Thamsborg 2001).

Because antimicrobials are prohibited in organic livestock production, however, bacteria in organic meat and poultry products are likely more susceptible to antimicrobials. Thus, dissemination of antimicrobial resistant bacteria may be curtailed, potentially contributing to maintaining the effectiveness of antimicrobials used in human and veterinary medicine. There is a paucity of data on antimicrobial resistant bacteria associated with organic food. The Denmark study reported by Heuer and others (2001) found that most *Campylobacter* isolates (>90%) from the organic and conventional flocks, neither of which used antibiotics for growth promotion, were susceptible to antimicrobials. Sato and others (2004a) compared the prevalence and antimicrobial susceptibility of *Campylobacter* isolated from organic and conventional dairy herds in Wisconsin and reported no significant difference between the production methods. Studies on *Salmonella* and *Campylobacter* in retail chicken carcasses obtained in the greater Washington, D.C. area indicated that more carcasses of organically produced chickens were contaminated with *Salmonella* than carcasses of conventionally produced chickens (61% compared with 44%, respectively), while *Campylobacter* contaminated 76% of the carcasses of organically produced chickens and 74% of the carcasses of conventionally produced chickens. The majority (80%) of *Salmonella* Typhimurium isolated from 19 carcasses of organically produced chickens were susceptible to 17 antimicrobials tested, whereas all *Salmonella* Typhimurium isolates from carcasses of 12 conventional chickens were resistant to at least five antimicrobials. A significant difference in ciprofloxacin resistance was also observed in *Campylobacter* recovered from organic and conventional chickens. Less than 5% of *Campylobacter* isolates from organic chickens were resistant to ciprofloxacin, whereas 20% of the conventional chicken counterparts were resistant to the drug. *Staphylococcus* isolated from organic dairy herds (Tikofsky and others 2003) and bulk tank milk (Sato and others 2004b) was also shown to be more susceptible to antimicrobials than their counterparts from conventional dairy herds. However, clear differences in multiple drug resistance in poultry have been reported (Luangtongkum and others 2006). Of 694 isolates of *Campylobacter* from organic broilers and turkeys, less than 2% had resistance to fluoroquinolones compared with 46 to 67% of conventionally raised broilers and turkeys.

These studies indicate that the prevalence and antimicrobial susceptibility of foodborne pathogens varies among different animals and production systems. Bacteria from organic animal production are generally more susceptible to certain antibiotics; however, the data from such a limited number of studies are inconclusive. Baseline data on the microbiological safety of organic foods are needed, as sales of organic foods are expected to increase (Sloan 2002).

Food manufacturing environments

Food antimicrobial agents and sanitizer. In contrast to genetically based resistance, bacterial adaptation to food sanitizers and preservatives is a transient state, and is thus very difficult to measure in vivo or in nonlaboratory settings. Among the few studies examining these substances, acquired resistance has been reported for benzoic acid or benzoates, sorbic acid or sorbates, and parabens. The studies examining potential acquired resistance to traditional regulatory-approved food antimicrobial agents are limited. Recent studies have examined potential resistance or adaptation in laboratory-type, food-like environments as well as cross-protection of resistant survivors to other stresses.

Sorbate and benzoate resistance. The primary application of benzoic acid and benzoates is to inhibit growth of yeasts and molds in acidic foods. Innate resistance of certain yeasts and molds to benzoates is a major cause of spoilage. Warth (1985) reported that a number of yeasts are capable of growing in the presence of approximately 500g/mL benzoic acid, including *Schizosaccharomyces pombe* and *Zygosaccharomyces bailii*. Others, including *Pichia membranefaciens* and *Byssochlamys nivea*, are also naturally resistant to benzoates (Chipley 1993).

Warth (1988) reported that when *Candida krusei, Hansenula anomala, Kluyveromyces fragilis, Kloeckera apiculata, Saccharomyces cerevisiae, Saccharomycodes ludwigii, S. pombe,* and *Z. bailii* were incubated with subinhibitory concentrations of benzoic acid, they had MICs to benzoic acid 1.4- to 2.2-fold higher than unexposed cells. Warth (1988) suggested that the resistance mechanism of yeasts pre-exposed to weak-acid preservatives, including benzoic and propionic acids, is related to membrane permeability and the ability of the cells to continuously pump preservatives out of the cell.

Studies to determine the effect of pre-exposure to sorbic acid on subsequent resistance have shown species-specific results. Warth (1977) observed that *Z. bailii* grown in the presence of sorbic acid displayed increased resistance upon subsequent exposure to the compound, compared to unexposed cells. Bills and others (1982) found that pre-exposure of *Saccharomyces rouxii* to sorbic acid significantly increased resistance to the agent, as seen in shorter lag times and/or shorter time to stationary phase, compared with previously unexposed cells. One mechanism for the resistance acquired by the yeasts is induction of an inducible, energy-requiring system that increases sorbic acid efflux (Bills and others 1982; Warth 1977). However, resistance of yeasts to sorbic acid and other weak acids probably involves more than one system (Brul and Coote 1999). Schroeder and Bullerman (1985), however, found little or no increase in the resistance of *Penicillium digitatum* or *P. italicum* when exposed to increasing levels of sorbic acid.

Moir and Eyles (1992) compared the effectiveness of methyl paraben and potassium sorbate on the growth of four psychrotrophic foodborne bacteria, *A. hydrophila*, *L. monocytogenes*, *Pseudomonas putida*, and *Yersinia enterocolitica*. Little or no adaptation was found to occur when cells were exposed to sub-inhibitory concentrations of antimicrobials.

Innate resistance to sorbic acid is demonstrated by catalase-negative LAB, *Sporolactobacillus*, some *Pseudomonas* spp., and other bacteria, *Brettanomyces*, *Candida*, *Saccharomyces*, *Torulopsis*, *Z. bailii*, and other yeasts, and *Aspergillus*, *Fusarium*, *Geotrichum*, *Mucor*, *Penicillium*, and other molds (Sofos and Busta 1993; Warth 1988). As with benzoic acid, some microorganisms can metabolize sorbic acid. Molds isolated from cheese, including 7 *Penicillium* spp., exhibited growth in the presence and degradation of 0.3 to 1.2% sorbate (Finol and others 1982). *Penicillium puberulum* and *P. cyclopium* were the most resistant species evaluated.

Parabens. The antimicrobial activity of parabens or alkyl esters of *p*-hydroxybenzoic acid increases with increasing length of the alkyl chain or increased hydrophobicity. Bargiota and others (1987) examined the relationship between lipid composition of *S. aureus* and resistance to parabens. Differences were found for total lipids, phospholipids, and fatty acids between *S. aureus* strains which were relatively resistant or sensitive to parabens. It was suggested that these changes could influence membrane fluidity and therefore affect adsorption of the parabens to the membrane. Russell and Chopra (1996) reported that deep rough mutants (heptose-less) of *Salmonella* Typhimurium and *E. coli* having exposed phospholipid were more sensitive to parabens than wild-type strains that had an intact lipopolysaccharide layer. Juneja and Davidson (1993) altered the lipid composition of *L. monocytogenes* through growth in the presence of added fatty acids (C14:0, C18:0, or C18:1) and saw a correlation between lipid composition of the cell membrane and susceptibility to antimicrobial compounds. Some microorganisms are capable of enzymatically degrading parabens (Russell and Chopra 1996). Valkova and others (2002) reported that strains of *Enterobacter cloacae* and *E. gergoviae* produced an enzyme controlled by the *prbA* gene that hydrolyzes the ester bond of parabens. They suggested that there is potential for a transfer system for the *prbA* gene among bacteria.

Lysozyme. Lysozyme is a naturally occurring enzyme that is approved by many regulatory agencies throughout the world for use in foods. Lysozyme is mostly active against Gram-positive bacteria, acting on the peptidoglycan component of the cell wall (Cagri and others 2004). Gram-negative bacteria are more resistant because they have a lipid bilayer outer membrane that acts as a barrier to prevent access of lysozyme to its target (Masschalck and Michiels 2003). Tamaki and Matsuhashi (1973) observed that *E. coli* mutants with an incomplete lipopolysaccharide (glucose residue-negative) membrane were sensitive to lysozyme.

Some bacterial species maintain the genetic machinery necessary for survival against lysozyme. *E. coli* is known to encode a lysozyme-binding protein that effectively inactivates the enzymatic activity of the compound (Deckers and others 2004; Monchois and others 2001). Many species are able to posttranslationally modify the constituents of their peptidoglycan walls to achieve resistance to enzymatic cleavage by lysozyme (Clarke and Dupont 1992; Zipperle and others 1984). Acetylation of the n-acetylmuramic acid at C6 (*O*-acetyl) and N-acetylation of n-acetylglucosamine of the peptidoglycan are both widespread among Gram-positive species and influence lysozyme resistance (Clarke and Dupont 1992; Masschalck and Michiels 2003; Weidenmaier and others 2003; Zipperle and others 1984). Some organisms also contain sigma factors (σ), such as σ^E, that upregulate stress response mechanisms against various environmental stresses, including lysozyme (Kallipolitis and others 2003). *Streptomyces coelicolor* mutants containing a σ^E deletion were shown to be up to 50 times more sensitive to lysozyme than were wild-type cells (Paget and others 1999a, 1999b).

Resistance to lysozyme may also be acquired. *Bacillus subtilis* mutants lacking bacilysin-production ability were demonstrated to be 200 to 300 times less resistant to lysozyme than wild-type cells (Ozcengiz and Alaeddinoglu 1991). Furthermore, following transduction of a bacilysin-encoding DNA fragment, mutants retained or acquired resistance to lysozyme equivalent to wild type cell resistance.

Plant-derived antimicrobials. Naturally occurring antimicrobial compounds obtained from plants include phytophenols, essential oils and their chemical components (some of which are phytophenolic) from spices and herbs, and sulfur-based compounds from onions, garlic, and cruciferous vegetables (Davidson 2001). As with previously discussed food-related antimicrobial agents, most research on resistance to these compounds has involved innate or intrinsic properties of target microorganisms.

B. cereus exposed to nonlethal concentrations of carvacrol, a component of the essential oils of oregano and thyme, demonstrated resistance to the normally bactericidal compound (Ultee and others 2000). Resistant cells had decreased cell membrane fluidity and changes in the phospholipid and fatty acid composition of the cell membrane.

Koga and others (1999) reported that certain strains of *Vibrio parahaemolyticus* are more resistant to basil and sage essential oils than their parent strain. In contrast, Ohno and others (2003) passed *Helicobacter pylori* through 10 transfers of lemongrass essential oil without any increase in resistance. Rickard and others (2004) exposed *E. coli* SPC105 to aqueous and ethanolic extracts of 9 different spices to determine growth inhibition and induction of the *mar* operon. Ethanolic extracts of all 9 spices inhibited growth of the microorganism, and cinnamon, tarragon, dill, garlic, cayenne pepper, and paprika induced the *mar* operon. The essential oil of the Australian tea tree (*Melaleuca alternifolia*), or tea tree oil (TTO), is inhibitory to a number of foodborne microorganisms. Gustafson and others (2001) found that mutants of *E. coli* AG100 exhibiting the Mar efflux phenotype were slightly more resistant to TTO than the parent strain. Longbottom and others (2004) investigated mechanisms of TTO-resistance among *P. aeruginosa,* finding that resistance is related to barrier properties of the outer membrane as well as efflux capabilities.

Bacteriocins. Nisin is an antimicrobial peptide (bacteriocin) produced by *Lactococcus lactis* spp. *lactis* that is inhibitory toward many Gram-positive bacteria and approved around the world for use in many foods (Cleveland and others 2001). Nisin-resistant isolates are generated from vegetative cells of *S. aureus, Bacillus licheniformis, B. subtilis, B. cereus* (Ming and Daeschel

1993), and *C. botulinum* (Mazzotta and Montville 1997) at similar frequencies. Nisin resistance in *L. monocytogenes* occurs at a frequency of 10^{-6} (Harris and others 1991; Ming and Daeschel 1993). Membranes from nisin-resistant *L. monocytogenes* isolates have decreased fluidity, presumably limiting or reducing the ability of nisin to penetrate the membrane (Ming and Daeschel 1993).

The major limitation of nisin use in food products may be the development of nisin-resistant strains (Harris and others 1992), which has been reported for other bacteriocins. For example, when pediocin Ach (PA1) is used to inhibit *L. monocytogenes*, the preservation system ultimately fails when pediocin-resistant cells grow out (Motlagh and others 1992). If resistance to specific bacteriocins were conferred by unique mechanisms the problem could be easily overcome by the use of multiple bacteriocins (Hanlin and others 1993). In addition, surface treatment of RTE meat products with nisin solutions induced an initial reduction of inoculated *L. monocytogenes* cells, but allowed multiplication of survivors during subsequent storage. When the nisin treatment was followed by exposure to acetic or lactic acid or potassium benzoate solutions, however, bacteriostatic and bactericidal effects were observed during product storage (Geornaras and others 2005; Samelis and others 2005b).

Sanitizers and disinfectants. Although sanitizers, disinfectants, and sterilants are not intentionally incorporated into finished food products, resistance to them may confer cross-resistance to some antibiotics. The long-term effects of extensive sanitizer use in food processing environments on the characteristics of resident microflora has been the subject of much debate. Many investigators in this area have applied techniques used in antibiotic resistance studies, such as the use of MICs, to the study of these biocides. But doing so may be a serious limitation because in most of these studies the MIC level determined to be resistant is as much as 10- to 100-fold lower than the level of biocide used in actual practice.

In contrast to antibiotics, which inhibit a specific biosynthetic cellular target, most biocides employed in the food industry attack multiple, concentration-dependent targets, causing major cell wall and membrane damage in a relatively short time (Russell 2003a). Thus, mutations resulting in antibiotic resistance are much more likely to occur than mutations resulting in acquired resistance to biocides. Some researchers (Aase and others 2000; Lunden and others 2003; Medrala and others 2003) have suggested that persistence of some bacteria in the food processing environment can be associated with sanitizer resistance, while others (Earnshaw and Lawrence 1998; Heir and others 2004a; Holah and others 2002) have discounted such a relationship.

Because common food plant sanitizers are more effective against planktonic cells than cells in biofilms (Stopforth and others 2002), the apparent resistance of biofilms to sanitizers is a concern. Biofilms are exopolysaccharide matrix-encapsulated bacterial cells which adhere to each other and to surfaces. Biofilms are considered as microcolonies or clusters of cells enclosed within a hydrated matrix, with pores or channels throughout their structure. The exopolysaccharide matrices form an extensive network, facilitating the initial attachment of cells, formation and maintenance of the biofilm structure, increased resistance of the biofilm to environmental stress and sanitizers, and nutrient capture. Cells in biofilms may exhibit increased resistance to antibiotics, which may stem from a number of factors—presence of a glycocalyx matrix preventing antimicrobials from accessing bacterial cell surfaces (Cloete 2003); chemical interaction between the disinfectant and the biofilm itself; modulation of the microenvironment; production of degradative enzymes (and neutralizing chemicals); or genetic exchange between cells in a biofilm (McDonnell and Russell 1999). Further, Cloete (2003) reported that cells in biofilms have the potential to genetically adapt to antimicrobial biocides, such as sanitizers, through mechanisms such as the *mar* operon. The parallels between mechanisms of resistance to antibiotics and organic acids and bacteriocins are shown in Table 7. Concern over potential development of sanitizer resistance has led some food processors to practice sanitizer rotation. However, Lunden and others (2003) found that adaptation among related and unrelated disinfectants was nonspecific; therefore, rotation may be of questionable effectiveness.

QAC-based sanitizers and disinfectants have been used globally in food manufacturing facilities for decades. Resistance among staphylococci to low levels of QACs has been reported in isolates from clinical and food processing environments (Heir and others 1999). Resistance to QACs in clinical strains of staphylococci appears to be encoded by 1 of 3 separate MDR determinants—*qac*A, *qac*B, and *qac*C (Heir and others 1995). The *qac*A/B family confers broad resistance and is predominantly located on the large (19–30 kb) plasmids, but has also been found on the chromosome of clinical *S. aureus* isolates (Gillespie and others 1989). Transfer of resistance from coagulase-negative

Table 7—Examples of bacterial resistance mechanisms

Mechanism	Action	Antibiotics	Organic acids	Bacteriocins
Export	Specific	Tetracycline Phenicols	F_0F_1 ATPase pumps out protons, anions	Not applicable, bacteriocins not in cytoplasm
	Non-specific	Organic solvent tolerance	Accumulate intracellularly	
Destruction	Specific or general	β-lactamases and cephalosporinases	Not applicable	Protease, specific "bacteriocinase"
Modification	Specific	Acetylation, adenylation, methylation, or phosphorylation of aminoglycosides Acetylation of phenicols	Not applicable	Dehydroreductases can inactivate lantibiotics such as nisin
Altered receptors	Specific	Penicillin binding proteins Ribosome DNA gyrase RNA polymerase	No receptor required	Probable, but not reported to date
Membrane composition	General	Altered membranes in resistant *E. coli* and bacilli	May affect permeability	Demonstrated for nisin resistance

staphylococci to enterotoxin producers is also a concern. Heir and others (1995) demonstrated that resistance plasmid pST827 or related plasmids are widespread in staphylococci isolated from the food processing environment. They reported that the *qac*A-C resistance determinant genes occurred among strains isolated from food contact surfaces in three separate meat and poultry processing facilities and *qac*A/B genes were found in staphylococci isolated from bakery products.

Acquired QAC resistance in *S. aureus* is directly related to the efficiency of efflux pump systems. This same proton motive force-driven multidrug efflux pump appears to be present in some *L. monocytogenes* strains (Aase and others 2000). Sensitivities of 19 *L. monocytogenes* isolates, including 5 strains linked to a large outbreak from consumption of deli meats and hot dogs, were evaluated against several sanitizing compounds used in the meat industry (Romanova and others 2002). Five strains exhibited resistance to a commercial QAC, myristalkonium chloride, and BC, while all others were either sensitive or intermediate in resistance. Three of the 5 resistant strains also were resistant to hydrogen peroxide, but none of the strains was resistant to hypochlorite. All of the QAC-resistant strains contained two plasmids, although the presence of the large plasmid was not correlated with resistance. However, these researchers discovered that the *mdr*L gene can be both chromosomal and plasmid-borne.

Characterization of the resistance of *Listeria* isolates from food, human, and environmental sources revealed that QAC resistance was related to the presence of a plasmid that readily transfers among *Listeria* spp. and between *L. monocytogenes* and *S. aureus* (Lemaitre and others 1998). The *Listeria* spp. showing resistance probably harbored a plasmid conferring high-level resistance to multiple disinfectants, and the strains may have a *qac*A–*qac*B complex similar to that in *S. aureus*. Resistance to BC was defined as an MIC of 16 ppm. Interestingly, only 11.5% of 26 total clinical *L. monocytogenes* isolates were resistant, while 19% of 42 total foodborne isolates, including all 6 found on poultry carcasses, were resistant. The study confirmed high transfer rates of antimicrobial resistance-coding plasmids among members of the genera *Listeria* and *Streptococcus* or *Enterococcus,* as well as between *Listeria* and *S. aureus*. Although QAC resistance in *L. monocytogenes* food processing isolates is more common than in clinical isolates, no correlation was found between resistance and pulsed-field gel electrophoresis (PFGE) profile nor persistence in the environment (Heir and others 2004a). Such findings could suggest an adaptive mechanism to obtain resistance, or environmental selective pressure.

While there are a number of other sanitizers and disinfectants used industrially, resistance to them has rarely been characterized. Bacterial isolates from ice cream and poultry manufacturing facilities were found to have varying levels of sensitivity to QAC, tertiary alkylamine, potassium persulfate, and sodium hypochlorite (Lunden and others 2003). The authors observed adaptation to QAC and tertiary alkylamine after 2 h of sublethal exposure; the highest MIC increase observed was 3-fold. Progressive increases in disinfectant concentration during incubation resulted in increased resistance against all substances except potassium persulphate. They reported that cross-adaptation among disinfectants occurred regardless of differing mechanisms of action. The authors concluded that persistent strains are generally more resistant to sanitizers than transient strains and that rotation of sanitizers may prove ineffective because of cross-adaptation. Of note, however, in the investigation, biocides such as hypochlorite and QAC were added to nutrient broth, which would tend to neutralize the biocides. Heir and others (1995) and Sundheim and others (1992) observed some QAC resistance in Norwegian meat and poultry facility staphylococcal isolates, including one *S. aureus* isolate. The level of resistance in pure culture, however, was below recommended QAC use concentrations and may be of minimal practical importance. The study also demonstrated increased resistance to BC, a common component of commercial QAC products, following subculturing in the presence of the disinfectant. The enhanced resistance appeared to be retained upon further subculturing in the absence of BC, and enhanced the ability of some strains to survive sanitizer suspension tests at 150 and 200 ppm.

Compared to other sanitizers and disinfectants, bacterial resistance to QACs is the most studied and is of greatest concern. While resistance is documented, the levels of use of QACs and other agents typically exceed the MICs of "resistant" organisms, making resistance of minimal concern with respect to food safety.

Antibacterial products for the home

In addition to use of sanitizers during food manufacturing, various cleaning products, some of which make a hygiene claim, are used by consumers. Induction of the *mar* operon by various household items, including herbs and spices, food and beverages, and household cleaning products, was assessed by Rickard and others (2004). Bath foam, hair gel, a general cleaner, fabric softener, and 1 mM sodium salicylate strongly induced the *mar* operon in *E. coli* SPC105. An antibacterial spray cleanser, antibacterial dishwashing liquid, regular dishwashing liquid, and triclosan (10 μg/mL) inhibited growth of *E. coli* SPC105, without inducing the *mar* operon. It is not known whether normal triclosan usage levels would induce this same effect.

Aiello and others (2005) examined whether household use of antibacterial cleaning and hygiene products is an emerging risk factor for carriage of antimicrobial drug-resistant bacteria on hands of household members. They found that antibacterial product use did not lead to a significant increase in antimicrobial drug resistance after a year, and that it did not have an effect on bacterial susceptibility to triclosan. But, they said, more extensive and longer term use of triclosan might provide a suitable environment for emergence of resistant species and that further research on the issue is needed. McBain and others (2003) studied the effect of continuous triclosan dosing at commercial handsoap product levels in a simulated drain microcosm environment. The results indicated no effect on the bacterial community susceptibility profile to test biocides or antibiotics, including triclosan itself. The authors concluded that the emergence of antibiotic resistance through TCS use in the kitchen is highly improbable.

Lear and others (2002) studied potential development of resistance to PCMX and TCS in an industrial setting. The industrial environment chosen was the laboratory and factories of two biocide manufacturing companies. Environmental sites chosen in these settings were those with likely regular exposure to PCMX or TCS. The authors concluded that the presence of residual biocide concentrations in these industrial environments did not promote the emergence of bacterial tolerance or resistance.

More specifically, addressing triclosan, Russell (2004) reported that while triclosan resistance in laboratory experiments may be associated with changes in antibiotic susceptibility, comprehensive environmental surveys have not demonstrated any association between triclosan usage and antibiotic resistance. Several others (Gilbert and McBain 2003; IFH 2003) have concluded that there is no equivocal evidence that biocide usage contributes to the development of antibiotic resistance either in clinical practice or in the general environment. Russell (2004) pointed out that triclosan has several important uses, and the future aim must be to retain these applications while eliminating the more frivolous and unnecessary ones. Levy (2001) urged prudent use of these products.

Risk Factors for Human Infection by Antimicrobial Resistant Foodborne Pathogens

Evidence linking antimicrobial use in food animals to human health risk points to but does not prove a human health threat (Barza and Travers 2002). The controversy about the contribution of antimicrobial use in food animals to resistance among antimicrobials that are clinically important in human medicine is fostered and sustained by the inability to obtain direct, quantitative information about the magnitude and nature of the contribution (Lipsitch and others 2002). It would help solve the controversy if data were available demonstrating that there are more frequent or severe infections or increased morbidity or mortality, than would exist otherwise as a result of food animal-to-human transfer of antimicrobial resistance.

There are several ways in which antimicrobial resistance in foodborne pathogens may create an added public health burden. The biggest risk factor for human infection by antibiotic-resistant foodborne pathogens is the very existence of such resistant organisms. If one accepts their existence, the most frequently identified risk factor for infection with antibiotic-resistant bacteria is prior antibiotic exposure. Other risk factors for acquiring antibiotic-resistant foodborne infections are essentially the same as those for acquiring infections with antibiotic susceptible foodborne pathogens.

IFT's Expert Report on *Emerging Microbiological Food Safety Issues: Implications for Control in the 21st Century* includes a thorough discussion of factors that affect host susceptibility to infectious diseases in general, and foodborne diseases in particular (IFT 2002b). Long recognized risk factors for infectious diseases in general include age (less than 5 or greater than 50 years of age), pregnancy, immunosuppression (due to chemotherapy, HIV infection, or other illness), and reduced liver or kidney function. People with HIV infection, for example, have been shown to be at higher risk for *Salmonella* (Celum and others 1987; Gruenewald and others 1994) and *Shigella* (Baer and others 1999) infections, and to be more likely to develop invasive disease. The relative risk for acquiring antibiotic-resistant versus susceptible infections in such higher risk populations remains unclear, but it is a reasonable assumption that the risk of treatment failure in immunosuppressed individuals with antibiotic-resistant microbial infections would be elevated. Risk factors for infection with foodborne pathogens include all of the factors described above, as well as decreased gastric acidity (often due to antacid use) and other factors (such as consumption of fatty foods or large volumes of liquid) that may protect bacteria from stomach acid.

It is possible that resistance to antimicrobials used in food animal production may result in the spread of antimicrobial resistant pathogens among food animals, thus increasing the potential for human exposure to these pathogens. Very few studies have evaluated risk factors for acquiring antibiotic-resistant versus susceptible infections with the same microorganism. Kassenborg and others (2004) found that people with domestically acquired fluoroquinolone-resistant *Campylobacter* infections were 10 times more likely than healthy controls to have eaten chicken or turkey cooked at a commercial establishment. These findings are very similar to those of Friedman and others (2004) for all (resistant or susceptible) *Campylobacter* infections; thus, they do not seem to be unique risk factors for resistant infections. Kassenborg and others (2004), however, determined that travel outside the United States is a risk factor for fluoroquinolone-resistant *Campylobacter* infections compared with fluoroquinolone-susceptible infections. Interestingly, the authors did not find that prior use of fluoroquinolones was a risk factor. (Patients with fluoroquinolone-resistant infections were not more likely than patients with susceptible infections to have taken fluoroquinolones in the month before the stool specimen was collected.)

Additionally, it is possible that people taking antimicrobials for reasons other than a foodborne illness may be at increased risk of acquiring an infection with a resistant organism. Many lines of evidence suggest this is the case. The increased risk of infection with antibiotic-resistant foodborne pathogens in people taking antibiotics for other reasons has been recognized for more than 20 y. The basis for this increased risk is believed to be the disturbance of the commensal microflora and epithelial surfaces of the intestinal tract which normally confer a barrier or protective layer against colonization and infection by exogenous organisms. Antibiotic use causes a transient decrease in an individual's resistance to colonization by noncommensal bacteria and increases the potential of infection upon exposure to foodborne pathogens (Anderson and others 2003; Angulo and others 2000). During administration of antibiotics and a period afterward, the individual may have enhanced vulnerability to infection by intestinal pathogens. This can be due to a lowering of infectious dose. The belief that increased risk of infection results from suppression of normal flora is supported by two decades of streptomycin use in animal models to reduce the normal gut flora and render animals more susceptible to colonization with enteric pathogens (Myhal and others 1982).

Glynn and others (2004) compared risk factors for MDR versus pansusceptible infections with *Salmonella* Typhimurium. In this study, MDR was defined as resistance to at least ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (the ACSSuT phenotype). People with MDR-*Salmonella* Typhimurium infections were nearly 20 times more likely than people with susceptible infections to have received one of the ACSSuT drugs in the 4 w prior to illness. Eating turkey prepared in the home was only a modest risk factor in both univariate and multivariate analysis.

In a case-control study of *Salmonella* Newport infections, Varma and others (2004) reported that patients with MDR infections were more likely than patients with susceptible infections to have taken in the 4 w before illness a drug to which MDR-*Salmonella* Newport strains are resistant. These patients were also more likely to have eaten Mexican style cheese, cilantro, and fish. None of these associations was particularly strong, however. International travel was not identified as a risk factor for infection with MDR-*Salmonella* Newport.

The evidence strongly supports the suggestion that antibiotic resistance increases human infections by increasing the risk of infection in people who have had prior antibiotic exposure. The "attributable fraction" reflects the proportion of all infections that would not have occurred in the absence of recent or concurrent treatment with an antimicrobial to which the bacterium was resistant (Barza and Travers 2002; Cohen and Tauxe 1986). Also called "excess cases," this phrase describes the mechanism by which a person develops an infection as a result of use for unrelated reasons of an antimicrobial to which the inciting pathogen is resistant (Barza and Travers 2002). Although the magnitude of this increase cannot be known with certainty, Barza and Travers (2002) estimated that an additional 29379 nontyphoidal *Salmonella* infections and 17688 *C. jejuni* infections occur each year in the United States due to this increased risk.

It is worth noting, however, that trends in the prevalence of antimicrobial resistance in a particular microorganism do not necessarily reflect trends in the incidence of antimicrobial resistant infections. If the prevalence of resistance to a particular antibiotic in a pathogen doubles, but the incidence of infection is reduced by 50%, the incidence of antibiotic-resistant infections with that pathogen has not changed. As illustrated in Table 8, the incidence of many foodborne illnesses has declined in recent years, and in

Table 8—Changes in foodborne illness incidence and corresponding changes in antimicrobial resistance

Year(s)	Microorganism	Case rate[a]	Relative decrease or increase[b]	% resistant	Case rate[a]	Relative decrease increase[b]
1996–98	*Salmonella*	15.9		28% (2 or more antibiotics, 1996)	4.5	
2004	*Salmonella*	14.5	8% decrease	18% (2 or more antibiotics, 2003)	2.6	42% decrease
1996–98	*Salmonella* Typhimurium	4.9		34% (ACSSuT, 1996)	1.7	
2004	*Salmonella* Typhimurium	2.8	43% decrease	26% (ACSSuT, 2003)	0.7	59% decrease
1996–98	*Salmonella* Newport	1.2		8% (2 or more antibiotics, 1996)	0.1	
2004	*Salmonella* Newport	1.5	25% increase	25% (2 or more antibiotics, 2003)	0.4	300% increase
1996–98	*Campylobacter*	18.7		13% (ciprofloxacin resistance, 1997)	2.4	
2004	*Campylobacter*	12.7	32% decrease	18% (ciprofloxacin resistance, 2002)	2.3	4% decrease

[a]Per 100000.
[b]Decrease relative to the earlier measurement.

many cases, the incidence of resistant infections has declined as well. Nevertheless, the fraction of cases which can be attributed to prior or concurrent antibiotic use is the same.

Impact of Antimicrobial Use, Nonuse, and Resistance

Food manufacturing

Modifications of food product formulation and processing conditions to meet consumer demands for convenient, healthy, or "preservative-free" foods may concomitantly involve reduction of food preservation hurdle intensities and subsequently lead to sublethal stressing of microorganisms. As a result, surviving pathogens may have increased resistance and virulence and, thus, be more difficult to control; as a result antimicrobial hurdles may fail (Archer 1996; Samelis and Sofos 2003a, 2003b; Sheridan and McDowell 1998). During exposure to sublethal stresses, bacteria try to maintain their cellular integrity and homeostatic balance but the effort may lead to metabolic exhaustion and cell death, cell injury, or stress adaptation. Sublethal cell injuries may be repaired during product storage potentially leading to undesirable outcomes, while multiple cell injuries may lead to extended microbial lag phases and potentially cell death.

Spraying meat animal carcasses with organic acid solutions may lead to establishment of acid-resistant pathogens such as *E. coli* O157:H7, which may subsequently survive, colonize in the food manufacturing environment, and cross-contaminate subsequent batches of food. Studies have demonstrated that the potential may exist for survival and resistance development among *E. coli* O157:H7 in simulated environmental niches of meat plants where carcass decontamination interventions are applied (Samelis and others 2001a, 2001b, 2002a, 2002b, 2003, 2004a, 2004b, 2005b). Acid-adapted cultures (developed through growth in glucose-containing broth) inoculated on beef carcass samples were found more resistant than control cultures upon exposure to simulated spray-chilling with water or chemical solutions (that is, lactic acid, cetylpyridinium chloride [Stopforth and others 2004b]). Acid-stressed cultures of *E. coli* O157:H7 and *L. monocytogenes* were found capable of forming biofilms of increased resistance to sanitizers on stainless steel coupons exposed to meat decontamination runoff fluids (Stopforth and others 2002, 2003a, 2003b). The potential may also exist for changes in the microbial ecology and spoilage patterns of meat products treated with acidic solutions (Samelis and Sofos 2003a, 2003b). However, it is difficult to conduct in vivo studies in actual food environments in order to prove or disprove such hypotheses.

It may be hypothesized that stress-resistant pathogens may have played a role in the involvement in foodborne illness of traditionally low risk foods, such as fruit juices, fermented meats, fresh produce, and dried products. Irrespective of such concerns, it is notable that decontamination treatments are effective in reducing microbial contamination of carcasses and in helping meat processors meet regulatory performance standards and industry specifications. Potential strategies for minimizing the development of stress resistance may involve continuous application of lethal levels of preservatives; rotation of antimicrobial interventions; or optimization of the type, intensity, and sequence of interventions, to maximize microbial destruction and minimize resistance development (Samelis and Sofos 2003a).

Strategies for pathogen control based on multiple or single hurdles need to consider prevention of microbial adaptation and resistance development or selection, and should be designed to control potentially resistant and stress-adapted microorganisms. In all uses, selection of hurdles, their intensities, and application sequence should aim to maximize microbial control while avoiding pathogen stress adaptation or selection of resistant cells. The goal should be to apply hurdles of proper intensity in the appropriate sequence in order to metabolically exhaust cells through energy depletion during their efforts to repair injuries and maintain homeostasis. If such a strategy is properly developed and applied, surviving cells exhausted from initial stresses may be left without sufficient energy reserves to cope with subsequent stresses or the final gastric stress (Samelis and Sofos 2003a, 2003b).

Human health

The implications for human health and food safety of genetic exchange between animal, food, and medical microbial isolates are beginning to be explored (Teuber and others 1999).

Perreten and others (1997) demonstrated that a starter culture strain of *L. lactis* ssp. *lactis* was found to have collected genetic information from 4 other species of pathogenic and nonpathogenic bacteria associated with food environments. It is likely, they said, that *E. faecalis, S. aureus, L. monocytogenes,* and *E. coli* were sources for the antimicrobial resistance genes associated with the plasmid occurring in *L. lactis* ssp. *lactis*. The broad range of some plasmids and the action of transposons in many bacteria allow antibiotic resistance genes to transfer by conjugation between different species and genera.

Studies of the development of antimicrobial resistance in animals and transfer to humans have focused on use of antimicrobials for growth promotion. There are some limitations in these studies that warrant consideration. Generally, there is a lack of standardization among studies, significant differences exist in populations evaluated, differences exist in culture sample types and methods used to collect and culture pathogens, and

variations exist in the methods and definitions for determining resistance. Further, the majority of studies did not evaluate the effects of other risk factors that may influence the development of bacterial resistance. Until definitive, standardized methods are devised and applied to the study of antimicrobial resistance among human, plant, and animal isolates, these limitations must be taken into consideration when evaluating study results.

Once foodborne illness with an antibiotic-resistant pathogen does occur, the impact on human health may be manifest in loss of treatment options or treatment failure. Most *Salmonella* infections do not require antimicrobial treatment. However, it has been reported that 40 to 50% of patients with salmonellosis are treated with antimicrobial agents (Cohen and Tauxe 1986; Glynn and others 2004; Lee and others 1994). Antimicrobials are not indicated for uncomplicated *Salmonella* gastroenteritis because antimicrobial treatment does not reduce the duration or severity of symptoms, may prolong recovery and the carrier state, and increases the likelihood of the emergence of antimicrobial-resistant organisms.

Although treatment is not usually required for recovery from uncomplicated salmonellosis, it is strongly indicated in cases of severe or invasive disease. What constitutes "severe" disease may be subjective, but *Salmonella* infections result in an estimated 16430 hospitalizations and 582 deaths in the United States each year (Mead and others 1999). While it is certainly rational to assume that treatment failure may contribute to this morbidity and mortality, the contribution may be difficult to measure. It is not possible to predict the clinical course of individual patients, regardless of whether they are treated with an appropriate antimicrobial agent. However, there are numerous reports indicating that treatment with an agent to which the infecting strain shows decreased susceptibility does contribute to poor outcome. Many of these reports are cited by Crump and others (2003) who called for reevaluation of fluoroquinolone breakpoints for *Salmonella* Typhi and non-Typhi *Salmonella*. Examples of probable treatment failure due to decreased fluoroquinolone susceptibility, resulting in two patient deaths in Denmark, were reported by Molbak and others (1999).

Ceftriaxone is the drug of choice for treating invasive enteric *Salmonella* infections in children; fluoroquinolones are not approved for use in children. Cases of ceftriaxone-resistant *Salmonella* have been reported from several countries (Bradford and others 1998; Fey and others 2000; Gazouli and others 1998; Hammami and others 1991; Pitout and others 1998). Most of the estimated 1.4 million *Salmonella* infections that occur each year in the United States are in children and the elderly (Mead and others 1999).

Although *C. jejuni* infections are less likely than *Salmonella* infections to result in invasive disease or death, there is some evidence of adverse consequences in patients with fluoroquinolone-resistant infections who are treated with fluoroquinolones. In a study of fluoroquinolone-resistant *C. jejuni* infections in Minnesota, Smith and others (1999) reported that patients infected with antibiotic-resistant *C. jejuni* who were treated with fluoroquinolones had a longer duration of diarrhea (an average of 10 in contrast to 7 d) than patients infected with fluoroquinolone-sensitive isolates. Similarly, Marano and others (2000) reported longer mean duration of diarrhea (8 in contrast to 6 d) in patients infected with fluoroquinolone-resistant *Campylobacter* strains; longer diarrhea duration occurred among patients who took a fluoroquinolone for their illness as well as those who did not.

There are also data supporting an increase in virulence of infections by fluoroquinolone-resistant *C. jejuni* among people not treated with an antimicrobial drug or antidiarrheal agent. In a multistate study of FoodNet sites, it was determined that diarrhea lasted longer (mean duration of diarrhea of 12 in contrast to 6 d) when campylobacteriosis was caused by a fluoroquinolone-resistant strain; the increased duration of illness in people with resistant infections was not a result of treatment failure. In contrast, however, Unicomb and others (2006) observed that diarrhea duration was similar for patients infected with fluoroquinolone-resistant strains and patients infected with sensitive strains of *C. jejuni* (median duration for both groups was 7 d). Unicomb and others (2006) noted the possibility that the larger sample size of the Nelson and others (2004) study raised the statistical power to enable detection of a difference.

Another way in which antibiotic resistance may contribute to the burden of illness associated with foodborne pathogens is the potential for increased virulence of resistant strains. The relationship between antibiotic resistance and the apparent virulence of intestinal pathogens has been integrated in several studies, some of which are discussed below. Data for both nontyphoidal *Salmonella* and *Campylobacter* infections suggest that antimicrobial resistant strains of these bacteria are somewhat more virulent than susceptible strains (Barza and Travers 2002). However, some believe that increased virulence of antibiotic-resistant *Salmonella* has not been well characterized (Helms and others 2002).

As early as 1987, data from CDC outbreak investigations of community-acquired and nosocomial outbreaks of nontyphoidal *Salmonella* in the United States between 1971 and 1980 showed higher death rates in *Salmonella* outbreaks due to drug-resistant strains than drug-susceptible strains (Holmberg and others 1987). In a more recent CDC study in which nontyphoidal *Salmonella* infection was confirmed by culture, individuals with infections caused by MDR-microorganisms tended to be ill and were significantly more likely to be hospitalized and experience longer periods of hospitalization than those with antimicrobial susceptible infections (Lee and others 1994). Neither of these studies accounted for possible differences in virulence among *Salmonella* serotypes, and neither study controlled for patient age, both of which are possible confounding factors. A more recent study reviewed FoodNet and NARMS data between 1996 and 2001, and controlled for these and other factors. Resistance was again found to correlate with increased illness severity; *Salmonella* isolates resistant to at least 1 antibiotic agent were more frequently isolated from blood than were susceptible strains (Varma and others 2005).

Martin and others (2004) investigated the burden of illness associated with *Salmonella* Typhimurium infections in Canada, finding an increased hospitalization rate associated with isolates having the R-type AK/CSSuT than isolates susceptible to at least 1 of the agents. The authors estimated that 57% of hospitalized cases infected with *Salmonella* Typhimurium isolates having the AK/CSSuT phenotype and 72% of hospitalized cases infected with non-DT 104 isolates having the phenotype were attributable to the resistance pattern. Interestingly, in contrast to earlier reports, Wall and others (1994) did not find increased hospitalization rates associated specifically with DT 104 infections. It should be noted that Martin and others (2004) considered any isolates susceptible to kanamycin, chloramphenicol, or any agent in the AK/CSSuT group to be susceptible isolates. Therefore, isolates in which small genetic events that might have affected only 1 of the resistance genes, such as a small insertion or deletion, were placed in the same group as isolates that lost (or never possessed) the entire resistance cluster, and possibly other vital genes as well.

In a large study in Denmark, Helms and others (2002) determined the death rates associated with drug resistance in *Salmonella* Typhimurium through a matched cohort study. The authors linked data from the Danish Surveillance Registry for Enteric Pathogens with data from the Danish Civil Registration System, which includes data on all live-born children and citizens of Denmark, and data from the Danish National Patient Registry,

which contains data on all patients discharged from nonpsychiatric departments. They compared 2-y death rates for patients infected with *Salmonella* Typhimurium with a matched sample of the Danish population (adjusted for differences in comorbidity). Patients infected with pansusceptible *Salmonella* Typhimurium strains were 2.3 times more likely to die in 2 y after infection than the general population. The death rate for patients infected with *Salmonella* Typhimurium strains having the ACSSuT phenotype (mostly DT104) was 4.8 times that of the general population. For patients with quinolone-resistant infections, the death rate was 10.3 times higher than the general population. Since the authors did not have access to treatment data, it is impossible to assess the relative role of treatment failure versus possible increased virulence of resistant isolates in this study.

Antibiotic resistance in foodborne pathogens has clear human health impacts. Evidence strongly suggests that people who take antibiotics for other reasons are at increased risk of developing infections with antibiotic-resistant bacteria. Other risk factors may differ between outbreak-associated and sporadic illness, but the major risk factors for infection with resistant pathogens have generally been found to be similar to those for susceptible strains of the same organisms.

Other reports suggest that failure of therapy due to antibiotic resistance may result in longer duration of illness, more severe illness, or death, but it is difficult to evaluate the impact of treatment failure in an individual patient. Many recent studies also report an increased severity of illness associated with resistant infections, though the reasons are not entirely clear.

Trade

At the request of the U.S. Congress, the U.S. General Accounting Office (now known as the Government Accountability Office) produced a report that included information on how antibiotic use has affected trade (GAO 2004). The report notes that the United States and several of its key trading partners, such as Canada and South Korea, and its competitors, such as the EU, differ in their use of antibiotics in animals, such as the specific antibiotics that are permissible for the purpose of growth promotion. The United States, as well as Australia, Canada, Japan, and South Korea, allow the use in animals of some antibiotics from classes important in human medicine. However, Australia has reviewed risk assessments on virginiamycin and is currently reviewing tylosin to determine whether to continue to allow the use of these antibiotics for growth promotion. Canada plans to conduct similar risk assessments, and Japan is reviewing the use of all antibiotics for growth promotion. In contrast, New Zealand has completed risk assessments of antibiotics used for growth promotion, and no longer allows the use of any antibiotics for growth promotion that are related to antibiotics used in human medicine. The EU Commission has prohibited its member countries from using antibiotics in feed for growth promotion. However, the EU will still allow the use of coccidiostat and histomonostat drugs, which are feed additives that control parasites. No coccidiostat and most histomonostat drugs are used in humans.

The GAO report stated that according to officials of USDA's Foreign Agricultural Service, the Office of the U.S. Trade Representative, the U.S. Meat Export Federation, and the U.S. Poultry and Egg Export Council, to date, antibiotic resistance associated with use in animals has not been a significant factor affecting U.S. trade in meat products. Only Ukraine was identified in the report as having import requirements banning fresh or frozen poultry products from animals that were treated with antibiotics for growth promotion. Ukraine is not a significant market for U.S. poultry, however.

The presence of antibiotic residues in meat has had some impact on trade. In particular, Russia has previously banned U.S. poultry because of the presence of tetracycline residues. Japan established tetracycline residue tolerance at such a low level that extended withdrawal periods are required for swine destined for export to Japan. The U.S. officials reported that other issues have been more prevalent in trade discussions, including the use of hormones in beef cattle and animal diseases such as bovine spongiform encephalopathy and avian influenza.

Although Federal government and industry officials stated that antibiotic use in animals has not significantly affected U.S. trade to date, GAO found some indication that this issue might become a factor in the future (GAO 2004). Antibiotic use in animals could become a trade issue if certain countries apply their regulations on antibiotic use in animals to their imports. For example, use of antibiotics in the United States could become a trade issue with the EU because it stopped use of all antibiotics for growth promotion. However, the EU is not currently a significant market for U.S. meat because of trade restrictions, such as its hormone ban that effectively disallows U.S. beef.

The issue of antibiotic use in animals and the potential human health risk associated with antibiotic-resistant bacteria has also received international attention. Two joint Food and Agriculture Organization (FAO)/World Organization for Animal Health (OIE)/World Health Organization expert workshops were held in 2003[15] and 2004.[16] The WHO has been working on the issue of resistance as pertains to clinical and nonclinical use of antimicrobials and human health. The OIE has been addressing the issue as it relates to animal health, and during its 73rd general session adopted updated international standards on antimicrobial resistance (OIE 2005). In the Codex Alimentarius Commission (CAC), the FAO/WHO food standards setting organization, a code on minimizing and containing antimicrobial resistance was adopted in 2005 (CAC 2005) and it was agreed in principle to establish an intergovernmental task force to address the issue. The Commission agreed that any Codex work on the issue shall be based on sound science, follow risk analysis principles, have a clear focus on public health, and ensure a holistic approach to solving the issue.

Economic

There are essentially two main perspectives to the economic assessment of antibiotic resistance in food production. On the one hand, the costs for patients with antibiotic-resistant pathogens has been examined to some extent, and on the other, the economics of antibiotic use, or non-use, with no distinction on resistance status, in food animal production has also been estimated.

For antibiotic use in animals, the general approach to economic valuations has focused on the improved financial return to the producer on the use of a particular growth-promoting feed additive antibiotic that has claims for better feed efficiency or higher average daily gain. The rationale is that feed costs will decrease, as will time to market weight, and that there will be some benefit to overall animal health and welfare in the prevention of subclinical disease in the flock or herd. The savings (or return on investment) will be more than if the product were not used. In this general approach, the antibiotic resistance status of the bacteria in the animal is not factored in. The ultimate application of the economic valuation is to inform risk managers about the trade-offs that might be likely should the use of feed additives for growth promotion be discontinued.

[15] 1st workshop on non-human antimicrobial usage and antimicrobial resistance, Geneva, Dec. 1-5.
[16] 2nd workshop on non-human antimicrobial usage and antimicrobial risk management options, Oslo, Mar. 15-18.

Several reviews provide some specific insight into the complexity of developing economic models of use/non-use of feed additive antimicrobials in various food animal sectors. Early analyses dating from the 1970s can no longer be considered valid, due to significant changes in animal production technologies during the past three decades. Data from the 1990s are more contemporary; however, caution is necessary due to multiple changes during the past few years in areas such as consumer behavior (for example, food safety awareness campaigns, food animal infectious disease threats such as BSE, "antibiotic-free" marketing, "Atkins diet" fads), supply issues (for example, avian influenza effects), trade issues (for example, BSE, Foot and Mouth Disease threats), increased on-farm biosecurity, and food animal production industry consolidation.

The GAO (2004) identified and summarized recent studies that provided estimates of the potential economic impacts on producers and/or consumers of restrictions on antibiotics used in livestock production. Five of the 8 studies focused exclusively on swine production, but beef and poultry were also addressed in the remaining studies. Five of the 8 studies concentrated on U.S. production; 2 others included comparisons to Danish or Swedish production, and 1 was limited to Danish data.

Specifically, the studies estimated the economic effects of a partial and/or total ban of antibiotics used in food animals. The economic impacts on consumers and producers that were identified were generally comparable despite the use of a variety of economic models, assumptions about model parameters, and data sets. Overall, the studies concluded that a ban or partial ban on antibiotics in food animal production would increase costs to producers, decrease production, and increase retail prices to consumers.

The GAO (2004) cited an example in which the studies indicated that the elimination of antibiotic use in pork production could increase costs to producers from $2.76 to $6.05 per animal, which translated into increased consumer costs for pork from $180 million/year to more than $700 million/year.

An estimated increased consumer per capita annual cost of $4.84 to 9.72 (for all major meat types) was calculated on the basis of a partial to total ban on growth promoting feed additive antibiotic use in the United States (NRC 1999). Factors such as switching to non-antibiotic alternatives, cost, and further industry consolidation with fewer small family farms, were addressed. The swine industry has been the most analyzed food production sector. Beneficial effects of the use of growth promoting antibiotics were analyzed on an economic basis for reproductive efficiency, litter survivability, and feed utilization (Cromwell 1999). On a more generalized basis, a 10-y prospective analysis of a ban on feed additives for growth promotion for swine estimated that in the first year net profits (that is, cost per head finished) would decrease to $4.17, but would still amount to a $0.79 loss in the tenth year of a ban (Hayes and others 2002). Additionally Miller and others (2003) estimated that antibiotics used for growth promotion provide a 9% improvement in net profits.

In poultry, cost estimates are difficult to obtain; however, a long-term, multisite analysis found that there were variable effects over time in live weights, feed conversion, total condemnations, percent livability, and bird weight uniformity (Engster and others 2002). One study in cattle assumed that a partial (human use antibiotics only) versus a total ban on growth promoters would result in an increased price per pound of beef of 0.5 to 3.3% (Mathews 2002).

From these estimations it is clear that there is a negative economic impact on the removal of feed additives from food animal production; the largest financial impact is at the producer level. It is not clear that the increased costs borne by the consumer would be substantial. Antibiotic resistance and associated economic costs have not been fully analyzed with respect to foodborne diseases and the effects on food animal production.

Clearly, antibiotic resistance of pathogens emanating from use in both humans and animals has economic consequences as well as major human health consequences. In human medicine, the economic assessments of resistance seem to be nearly nonexistent, apparently owing to the diversity and breadth of the issues. One report cites "unpublished data from CDC" as estimating that in the early 1990s costs of antimicrobial resistance were $100 to 200 million, with related medical costs exceeding $4 billion (Cassell 1997). Also, in the mid-1990s, it was estimated that human antibiotic sales were more than $7 billion, with $4 billion directed toward nosocomial infections of antibiotic-resistant bacteria (John and Fishman 1997).

Miller and others (2006) noted that antimicrobial usage data may become economically important for reasons unrelated to animal productivity and animal health. The authors pointed out that with expanding global trade of animals and animal products, changes have occurred in restrictions and regulations associated with product movement, and future trade opportunities may be linked to antimicrobial usage.

Current economic studies on the use of antibiotics for control of bacterial diseases and fungicides (antimycotic agents) for fungal diseases in plants are lacking. Fewer or no antibiotics are expected to be available with the increased requirements on manufacturers of the Food Quality Protection Act (FQPA) of 1996, including a new safety standard of reasonable certainty of no harm that must be applied to all pesticides used on foods. EPA regulates the use of antibiotics and fungicides for plants in the United States. Analysis of profits versus the costs of reregistration of some antimicrobials may also deter companies from production.

Environmental

Phillips and others (2004) indicated that environmental considerations of antimicrobial uses in livestock are less striking than the economic considerations, noting that the increased demand for cropland as a result of decreased food efficiency without antibiotics could be met in the United States by an additional 2 million acres (USDA/NASS 2002). They added, however, that it can be argued that because of reduced feed efficiency, a ban on certain types of antibiotic uses in animal agriculture would increase animal waste per unit of animal product.

Very little is known about the exposure routes of antimicrobials into the environment (Halling-Sorensen and others 1998) or the fate and effects of antimicrobials on ecosystems (Baguer and others 2000; Gavalchin and Katz 1994; Halling-Sorensen and others 1998; Jorgensen 1984; Kummerer 2001a, 2003). The determination of risk from antimicrobials in the environment may be dependent on the respective biodegradability and adsorption in relation to the concentration, stability, and persistence of a drug in ecosystems as well as temperature and other environmental factors.

Most antimicrobials are water-soluble (tetracyclines, sulphonamides) and are excreted in urine as parent compounds (tetracycline and β-lactams) or metabolites (sulphonamides or macrolides) (Halling-Sorensen and others 1998). It has been estimated that 30% to 90% of a dose of an antimicrobial administered to humans and animals is excreted in urine as an active substance (Rang and Dale 1991). The same drug may be used in varied species and applications, resulting in different dosages and treatment durations, and wide-ranging environmental concentrations (Halling-Sorensen and others 2000). Concentrations of antimicrobials are normally found in the environment at significantly lower levels of magnitude than used therapeutically

Table 9—Environmental fate of biocides

Active biocide	Breakdown products	Environmental impact
Chlorine dioxide	Chloride and chlorate ions, or chlorite and chlorate	Minimal
Peracetic acid	Acetic acid, water, oxygen	Minimal to none
Peroxyoctonoic	Octanoic acid, water, oxygen	Minimal to none
Iodophor	Surfactant and iodine salt	Depends on surfactant
Acidified sodium chlorite	95% Cl	None to high dilution
Quaternary ammonium compounds	Mineralizations—readily to ultimately biodegradable	May affect waste treatment plants at high concentration

(Kolpin and others 2002; Kummerer 2003, 2004; Zuccato and others 2000). There are some differences among sanitizers with regard to their major breakdown products; these products and their qualitative effect on the environment are shown in Table 9.

Factors that determine antimicrobial movement and distribution include the chemical properties of the drugs and drug metabolites; extent of biological degradation in feces, sludge, soil, or water; propensity to separate in soil or water; and environmental characteristics such as temperature and soil type (Ingerslev and Halling-Sorensen 2001). Adsorption rates also differ among antimicrobials. The fate of antimicrobials released into the environment includes biodegradable mineralization to carbon dioxide and water, incomplete degradation and retention on sludge due to lipophilic properties, and metabolization to a more hydrophilic form of the parent lipophilic substance (Halling-Sorensen and others 1998, 2002, 2003).

Some antimicrobials present in soil and sediment can lose their antimicrobial properties as a result of binding to sediment particles or complex formation with ions (Kummerer 2004). However, there are contradictory results concerning lack of reduced antimicrobial activity and bioavailability due to adsorption or complex formation (Hansen and others 1992; Nygaard and others 1992). Mobility in the soil of a drug or metabolite through leaching determines whether the drug may impact the groundwater, terrestrial organisms, or aquatic organisms. Researchers have reported that antimicrobials may persist in sediment cores (Hektoen and others 1995; Jacobsen and Berglind 1998).

Fluoroquinolones strongly adsorb onto sewage sludge, soil, and sediments (Kummerer 2001b). In one study, over 99% of sarafloxacin, a fluoroquinolone that was formerly but is no longer approved in the United States to treat poultry diseases, persisted in soils for more than 80 days, theoretically due to its high ability to bind to soil (Marengo and others 1997). In a study of marine sediments, sarafloxacin was found in deeper layers of the sediment after 180 d at its initial concentration, with an estimated half-life in excess of 300 d. Eventual removal of the drug from the sediment was most likely the result of leaching and redistribution instead of degradation (Hektoen and others 1995).

Adsorption of oxytetracycline to solids was found to be negligible, in contrast to that for tylosin, the majority of which appeared to adsorb to the soil. Although the adsorption appeared to be reversible, there was the possibility that tylosin adsorption affected biodegradability (Ingerslev and Halling-Sorensen 2001). One study determined that metronidazole is moderately persistent in soil (Ingerslev and Halling-Sorensen 2001), and another demonstrated that 99.98% of the parent compound and its metabolites would be distributed in the water compartment (Macri and others 1988). Because metronidazole is both water soluble and relatively nonbiodegradable, it may also accumulate within ecosystems (Rang and Dale 1991).

Investigations of environmental effects of antimicrobials have most often been performed as acute toxicity tests in systems attempting to simulate biodegradation in natural ecosystems. As the fate and effects of these drugs are influenced by properties of respective aquatic or terrestrial ecosystems, test situations have been found to differ from natural conditions. Yet on the basis of present knowledge, the risks to human, animal, and environmental health from the direct impact of antimicrobials on bacteria in aquatic and terrestrial environments appears low. The American Academy of Microbiology concluded, however, that within a variety of interconnected ecosystems, antimicrobial agents can lead to drastic alterations in the biodiversity of affected ecosystems, reduction of microorganisms susceptible to the agents, and development of antimicrobial resistance (AAM 1999).

Several studies have used tylosin as a model to study the dispersion of antimicrobials via application of livestock manure onto soil and the potential impact of food animal antimicrobials on the environment. The studies have focused on tylosin, which is specifically active on certain bacteria, and presumably only has secondary effects on other groups of soil organisms (Muller and others 2002). In a controlled study in Denmark, within 2 w after tylosin was applied onto soil, the drug could not be detected, and within 3 w all degradation products had disappeared (Muller and others 2002). The drug did not reduce microbial diversity or system function and was thereby considered a "transient disturbance" from which the soil system function may eventually return to its former state (Muller and others 2002). Results of two other studies also demonstrated that tylosin did not have any significant effect at environmentally relevant concentrations (Baguer and others 2000; Muller and others 2002).

However, in a U.S. study, results suggested a link between the number and type of tylosin-resistant bacteria at agricultural sites using antimicrobials at sub-inhibitory levels compared to sites on which tylosin was not used (Onan and laPara 2003). The researchers noted, however, that limitations on their experimental design precluded them from excluding variables such as soil type and climate as significant factors in accounting for antimicrobial resistant bacteria.

Information from studies of aquatic environments shows that antimicrobials may be toxic for organisms other than the intended target bacteria (Baguer and others 2000). Cyanobacteria are the most sensitive algal species to be affected by antimicrobials in water systems (Halling-Sorensen 2000). Metronidazole was found to be relatively toxic to green algae, but did not have any direct acute effect on marine cocopeds and fish, which suggests the possibility of an indirect effect on algae (Lansky and Halling-Sorenson 1997). Furazolidine, used in fish farming outside the United States, is toxic to the mosquito larvae *Culex pipiens* (Macri and others 1998). However, the significance of the risks from contamination to the different aquatic and/or terrestrial organisms and ecosystems remains unknown.

Since the inception of the use of antibiotics for certain bacterial diseases in plants in the 1950s, no human health effects on applicators or harvesters with respect to infectious microbes have been documented (Vidaver 2002). Antibiotics that are not legally permitted for use in plant agriculture can contaminate plants and

have toxic or other growth and developmental effects. In studies of crop plants, sulfadimethoxine and enrofloxacin at highest concentrations depressed postgerminative development in all tested plants (Forni and others 2002), and flumequine depressed postgerminative development in weeds (Migliore and others 2000). The studies demonstrated that when plants are grown in areas contaminated with these and other antimicrobials, the storage of antimicrobials in plant tissues may result in the introduction of antimicrobials into the food chain (Migliore and others 2003). Fungicides for the control of plant diseases have been in use since the late 1800s; new fungicides, though few, are still being discovered and marketed because of the many and devastating fungal diseases of food and other plants (Agrios 2005). Plant viruses overcome plant resistance through a variety of mechanisms which are counteracted through cultural practices, resistant varieties, or insecticides targeting insect vectors.

The expression of virulence factors and the transfer of antimicrobial resistant bacteria and resistance genes are favored particularly by the presence of antimicrobials for a long period of time and at subinhibitory concentrations (Ohlsen and others 1998; Salyers and others 1995). Studies indicate that the conditions for transfer of resistance and the selection of resistant bacteria are not favorable at antimicrobial concentrations found in the environment (Summers 2002).

Overall, there is a demonstrable lack of knowledge and agreement about the frequency and extent of occurrence, fate, and effects associated with the antimicrobials entering the environment. As a result, it is difficult to assess the environmental impact of the use of antimicrobials without comprehensive knowledge of the use and fate of the drugs. The lack of data on the impact of the release of antimicrobials in the environment hinders appropriate risk assessment and management of the impact on human, animal, and environmental health of the use of antimicrobials in humans, animals, and on plants and resultant residues and resistance.

Management of Antimicrobials to Control Resistance

Responsible use

Guidelines exist for responsible (proper, appropriate, prudent, or judicious) use of antibiotics in veterinary and human medicine, and are similar in the medical and agricultural sectors (Phillips and others 2004). The guidelines are predicated on the assumption that use will sooner or later result in the development or expression of antibiotic resistance. The corollary that frames the prevention and control guidelines and activities is that voluntary or regulatory limitations on the overuse of antibiotics will lessen the development of antibiotic resistance and prevent further increase in resistance where already present.

Responsible use is not necessarily reduced use, however. In 2001, a U.S. Federal Interagency Task Force on Antimicrobial Resistance (USDHHS, AHRQ, HCFA, HRSA, USDA, USDOD, USDVA, EPA 2001) issued an action plan for four areas—surveillance, prevention and control, research, and product development. Defining appropriate antimicrobial drug use as that which "maximizes therapeutic impact while minimizing toxicity and the development of resistance," the Task Force noted that appropriate antimicrobial drug use should not be interpreted simply as reduced use, because the drugs offer valuable benefits when used appropriately. Further, in practice, this involves prescribing antimicrobial therapy when and only when it is beneficial to the patient, targeting therapy to the desired pathogens, and using the appropriate drug, dose, and treatment duration. It is overuse and misuse that must be decreased to reduce the selective pressure favoring the spread of resistance, the Task Force stated.

A substantial set of clinical guidelines, many of which are available from the CDC (2004a), has been developed for human medicine. These include recommendations for nosocomial infections (vancomycin-resistant enterococci, for example), malaria, sexually transmitted diseases, tuberculosis, and upper respiratory tract infections. Also, the Infectious Diseases Society of America cooperated with the Society for Healthcare Epidemiology of America to develop Guidelines for the Prevention of Antimicrobial Resistance in Hospitals (Shlaes and others 1997). Guidelines having a holistic approach for practitioners in several medical sectors were issued by the Alliance for the Prudent Use of Antibiotics (APUA 2006). Veterinary and animal producer organizations in many countries have also developed and implemented responsible use principles or guidelines. These address use in various species, including poultry, swine, dairy and beef cattle, and sheep. A number of organizations having such documents are listed in Table 10.

International organizations, such as the OIE, WHO, and the CAC, also have developed or are developing principles or codes of practice to contain antibiotic resistance. The WHO published Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (WHO 2000). The OIE issued 5 documents concerning antibiotic resistance, including Guidelines for the Responsible and Prudent Use of Antimicrobial Agents in Veterinary Medicine. The other 4 documents deal with risk analysis methodology, monitoring of use quantities, surveillance programs, and laboratory methodologies (Acar and Rostel 2001). Additionally, several Codex committees are addressing aspects of antibiotic resistance, including the committee on Residues of Veterinary Drugs in Foods (CCRVDF) and committee on Food Hygiene.

Most of the recommendations of the various guidelines can be summarized in three objectives: (1) emphasize actions to prevent disease, thereby eliminating the need for therapeutic use of antibiotics; (2) if a disease occurs in or threatens animals, consider methods other than antibiotic use to mitigate or prevent the effects of the disease; and (3) if antibiotics are necessary to prevent, control or treat a disease, first consider the use of antibiotics that are less important to human or veterinary medicine.

The guidelines mentioned above have substantial differences. Differences include target audience(s) (veterinary professional in contrast to an animal producer, for example), type of antibiotic use (for example, targeting therapeutic in contrast to growth promotion uses), and general (in contrast to specific nature of guidelines). There are common tenets in the various documents, however. Most have the dual aim of protecting human and animal health. They recognize that any use of antibiotics, human or animal, has the potential to select for antibiotic resistance. But they also recognize that all uses of antibiotics cannot be eliminated or severely constrained. Therefore, the intent of the documents is to promote appropriate use of antibiotics, maximizing efficacy and minimizing resistance development.

Far fewer guidance documents exist for responsible use of food antimicrobial agents, sanitizers, and other antimicrobials than for antibiotics. Regulations on food antimicrobial agent uses and limits are based on efficacy and human health impact of the agent itself. The recommended use levels do not consider the issue of resistance.

Responsible use guidelines recognize the unfortunate fact that little is known about the different conditions of use under which antibiotics may select for resistant bacteria. This leaves decision makers with the challenge of developing guidelines when the underlying, specific causes of antibiotic resistance are incompletely understood. But decision makers, including veterinarians and animal producers, cannot wait for the ultimate answer.

Table 10—Examples of responsible antibiotic use guidance documents

Source	Website or reference
Alliance for the Prudent Use of Antibiotics	http://www.tufts.edu/med/apua/
American Association of Avian Pathologists Guidelines to Judicious Therapeutic Use of Antimicrobials in Poultry	http://www.avma.org/scienact/jtua/poultry/poultry00.asp
American Association of Bovine Practitioners Prudent Drug Usage Guidelines	http://www.avma.org/scienact/jtua/cattle/cattle00.asp
American Association of Swine Veterinarians Basic Guidelines of Judicious Therapeutic Use of Antimicrobials in Pork Production	http://www.avma.org/scienact/jtua/swine/swine99.asp
American Veterinary Medical Association Position Statement and Principles for Judicious Therapeutic Antimicrobial Use by Veterinarians	http://www.avma.org/scienact/jtua/jtua98.asp
Australian Veterinary Association Code of Practice for the Use of Antimicrobial Drugs in Veterinary Practice	AVA (1999)
British Veterinary Association General Guidelines on the Use of Antimicrobials	BVA (1998)
British Veterinary Poultry Association Antimicrobials Guidelines	http://www.bvpa.org.uk/medicine/amicguid.htm
Canadian Veterinary Medical Association Guidelines for the Prudent Use of Antimicrobial Drugs in Swine	http://www.cvma-acmv.org/journals2.asp?sub=8
Federation of Veterinarians of Europe Antibiotic Resistance & Prudent Use of Antibiotics in Veterinary Medicine	http://www.fve.org/papers/pdf/vetmed/antbioen.pdf
National Cattlemen's Beef Association Producers Guide for Judicious Use of Antimicrobials in Cattle, National Cattlemen's Beef Association Beef Quality Assurance National Guidelines	http://www.bqa.org
National Pork Board Basic Guidelines of Judicious Therapeutic Use of Antimicrobials in Pork Production for Pork Producers	http://porkscience.org/documents/Other/psantibicprod.pdf
OIE Terrestrial Animal Health Code. Antimicrobial Resistance. Guidelines for the Responsible and Prudent Use of Antimicrobial Agents in Veterinary Medicine	http://www.oie.int/eng/normes/mcode/en_titre_3.9.htm
RUMA Alliance Guidelines—Responsible Use of Antimicrobials in Poultry Production	http://www.ruma.org.uk
RUMA Alliance Guidelines—Responsible Use of Antimicrobials in Pig Production	http://www.ruma.org.uk
RUMA Alliance Guidelines—Responsible Use of Antimicrobials in Dairy and Beef Cattle Production	http://www.ruma.org.uk
RUMA Alliance Guidelines—Responsible Use of Antimicrobials in Sheep Production	http://www.ruma.org.uk
World Veterinary Association/International Federation of Animal Producers/World Federation of the Animal Health Industry Prudent Use of Antibiotics: Global Basic Principles	http://www.worldvet.org/manuals/t-3-2.pru.doc

Alternative practices

Herd, flock, and other health management programs overseen by veterinary or other professionals attempt to minimize infectious disease outbreaks by using nonantibiotic interventions early in the life of the animals. The rationale is to promote healthy animals that do not become ill and are, thus, unlikely to be treated with an antimicrobial agent. Several current approaches are available. These nonantibiotic approaches have led to a need to establish performance standards for regulatory and commercial purposes (Rosen 2003). It should be noted that none of these "alternative" approaches can be used for therapeutic purposes as replacements for antibiotics.

Vaccines. Vaccines have been a key component of disease prevention for many years because they have many favorable attributes such as low cost, ease of administration, efficacy, multiple agent efficacy (viruses, bacteria, mycoplasma, and parasites, for example), and safety (worker, animal, environmental, lack of food residue). Adjuvants are sometimes included with vaccines to enhance the immune response. Various delivery systems or routes of administration (for example, muscle or in ovo injection, aerosol, topical, or oral [mucosal]) are used to administer the vaccine into the animal.

In the salmon and trout industries, vaccines against ERM and vibriosis have proven to be highly efficacious, and vaccination of young fish "fingerlings" is standard practice. Vaccines are also commercially available for use in the catfish and tilapia industries. The vaccines are usually applied to fingerlings by immersion, but in some cases (that is, vaccines for vibriosis in salmon and streptococcus in tilapia) injection is required (Klesius and others 2000). Probiotics and immunostimulants such as β-glucans are being used on a limited basis in aquaculture.

Future research in veterinary vaccine adjuvants will focus on particle delivery to antigen-presenting cells and immunostimulatory adjuvants to effect a higher and longer lasting state of immune response (Lowenthal and others 2000; Singh and O'Hagan 2003). New oral delivery systems, such as plant-based vaccines, are being developed that offer ease of administration, production, and other benefits, although the regulatory acceptance of these products remains to be clarified (Streatfield and Howard 2003).

Competitive exclusion. Direct-fed microbial products containing live microorganisms (known as probiotics) or products containing enzymes as the active ingredient are currently marketed in many countries (Anonymous 2006b). Probiotics, which contain one or more types of microorganisms and are administered orally, are currently approved for use in food animals in Europe and other countries, but as for the use of antibiotics for growth promotion, their mode of action is not fully understood. Probiotic bacteria could affect normal gut microflora by competitive exclusion of pathogenic bacteria, production of antibacterial products or enzymes that act on gut bacteria, or production of other metabolites that affect gut commensals (Reid and Friendship 2002; Simon and others 2001). Other approaches are the use of prebiotics

(nondigestible oligosaccharides) that permit beneficial gut bacteria to preferentially thrive, thus promoting overall host health (Mosenthin and Bauer 2000; Verstegen and Williams 2002). Supplementation of feedstuffs with phytase, an enzyme that allows greater host utilization of phosphorous, has also been advocated (Hatten and others 2001; Verstegen and Williams 2002).

Antimicrobial peptides. Antimicrobial peptides are host-cell-produced compounds that have been identified in plants, animals, and insects. Extensive research has led to an increased understanding of the mechanisms of action of porcine antimicrobial peptides, but has not addressed the numerous practical aspects that are necessary to achieve regulatory approval or marketplace success (Zhang and others 2000).

Bacteriocins. Pore-forming antibacterial proteins produced by microorganisms—bacteriocins—have been investigated for their potential use in the control of certain zoonotic pathogens in the avian intestinal tract (Joerger 2003). One bacteriocin, nisin, has been approved for use in several food products (Cleveland and others 2001).

Bacteriophages. Bacteriophages have been used successfully to prevent and treat bacterial diseases in humans and animals in Russia, but have failed to gain acceptance in Western countries owing to the focus on antibiotic use (Barrow 2001). They have also been used experimentally to control bacterial diseases in plants (Greer 2005). The possibility of using avian cytokines[17] as potential therapeutic agents has also been reported, but issues including dose and safety have not been resolved (Lowenthal and others 2000). As anti-infectives, bacteriophages have several attractive attributes including specificity, since each bacteriophage is directed toward a single kind of bacterium (although this results in a limited host range), lethality, projected low cost, and no residues in the food product (Greer 2005, Joerger 2003). However, questions surrounding the safety of using recombinant therapies, environmental containment, and phage resistance remain unresolved (Moldave and Rhodes 2003). As development of new antibiotics becomes less likely, interest in adapting bacteriophage therapy for plant and food animal applications may increase.

Alternative management practices. As noted in the National Pork Board's document—"Take Care: Use Antibiotics Responsibly"—antibiotics are only one part of an overall plan to maintain animal health (NPB 2005). The guidance discourages the automatic reliance on antibiotics without consideration of changes in management practices that may also address animal health issues. Several industries have found benefit in modifying practices as an alternative to antibiotic use.

Withholding feed. In the catfish industry, feeds medicated with sulfadimethoxine/ormetoprim 5:1 have an objectionable taste and catfish may not consume them as vigorously as standard feeds. Further, bioavailability of oxytetracycline is very low in catfish and individual fish must consume the proper amount of feed for a therapeutic dose to be obtained (Plakas and others 1988). Because of these problems, the current trend in the catfish industry involves withholding feed at the first sign of disease particularly when entering the "temperature window" for ESC disease and resuming normal feeding when water temperatures rise out of the permissive range. Farmers have found this technique to be almost as effective as administering medicated feeds, and the practice saves on the increased cost of medicated feeds.

Risk Analysis

Risk analysis has three components—risk assessment, risk management, and risk communication. An effective food safety system integrates science and risk analysis at all levels of the system, including food safety research, information, technology transfer, and consumer education (IFT 2002b). Risk assessment is the use of scientific data to identify, characterize, and measure hazards; assess exposure; and characterize risks. Risk assessment is currently being recommended as an important method for "science-based" decision making regarding food policy and antibiotic use in food animals. A thorough risk assessment can be a useful first step in the decision making process. It can provide a framework for the needed "big picture" view of a problem, its sources, and the consequences of proposed policy changes. However, risk assessment does not provide the necessary whole picture of an issue. Ideally, the entire system, including potential secondary effects, must also be considered in decision making. Additionally, the benefits, or the alternative risks of various risk management options, must also be evaluated. Due to the data requirements and uniqueness of bacterial and antibiotic interactions, analysis should be done on specific "bug–drug" combinations. Some of the decision analysis tools, as related to antibiotic use in food animals, are addressed below; a quantitative risk assessment of macrolide use in food animals is presented as an example.

When examining a potential policy change, it is critical to remember that a complex scientific phenomenon, such as resistance gene transfer, takes place within a much larger macrobiologic ecosystem and social system. Therefore, observance of similar resistance genes in food animals and humans does not explain the causal pathway of events or the flow of genetic information. An understanding of the system through good "shoe leather" epidemiology is essential (Phillips and others 2004).

Whenever a society is making a decision about a "risky" new technology, the negative impact or risk is only part of the equation. Rarely do people add extra risk to their lives unless there is some benefit. Most people agree that automobile travel is a risky practice; and, data support this view. Most of us, however, prefer the benefit of automobile use to that of alternatives, for example horse and buggy. The regulatory environment, however, is geared toward protecting the public from additional risk without consideration of benefits, hence the emphasis on risk assessment. Any objective risk assessment will show some risk, albeit very small. Within the context of the current U.S. regulatory framework, it is not possible for regulatory agencies, such as the FDA, to judge between the benefits of antibiotic use to livestock producers and risks to the public at large. Therefore, regulators must reject any practice that appears to produce any apparent risk unless a demonstrated higher risk would exist upon rejection of the practice.

For example, some evidence is accumulating, especially in the poultry industry, that there are significant human health benefits from antibiotic use to prevent or control food animal disease. It has been shown that subclinical disease levels of birds at slaughter significantly impact carcass contamination with pathogens such as *Salmonella* and *Campylobacter* (Russell 2003b). The levels of subclinical disease are reduced by antibiotic use. Therefore, the risk of antibiotic use is more than compensated for by a human health benefit. Cox and Popken (2004) conservatively estimated that at least 40000 illness-days/year are prevented by continued use of virginiamycin to reduce bacterial illnesses in chicken flocks. For every day of illness caused by continued antimicrobial use, an estimated 4000 excess illness-days are prevented. Similar results were recently reported for enrofloxcin and macrolide use in poultry (Cox and Popken 2006).

[17] cytokine: any of a class of immunoregulatory proteins (as interleukin, tumor necrosis factor, and interferon) that are secreted by cells especially of the immune system (definition from *Merriam Webster's Medline Plus*: www.nlm.nih.gov/medlineplus/mplusdictionary.html)

Case study: Overview of Macrolide Risk Assessment

The current regulatory environment strongly infers that "science-based" decision making utilizes risk assessment (Snary and others 2004). FDA's Center for Veterinary Medicine (CVM) issued a guidance document advising veterinary drug sponsors of one potential process for conducting a qualitative risk assessment of drug use in food animals (FDA/CVM 2002). Using this guideline, a quantitative deterministic model to assess the risk associated with two macrolide antibiotics—tylosin and tilmicosin—was developed. Tylosin is administered via medicated feed, drinking water, or injection to poultry, swine, and cattle to treat, prevent, or control disease, and enhance growth performance. However, not all routes of administration or claims have been approved in the United States for each species. Tilmicosin, a semisynthetic derivative of tylosin, is approved for treatment and control of respiratory disease in cattle and swine. The scenario presented below is discussed in more detail in the assessment publication by Hurd and others (2004), which sought to advance and inform the public debate regarding the use of food animal antibiotics.

The basic framework provided by the CVM guidance document was used to conduct a quantitative risk assessment of two macrolide antibiotics, tylosin and tilmicosin. Although other antimicrobial agents in the macrolide-lincosaminide-streptogramin B (MLSB) class, including lincomycin and virginiamycin, exhibit some cross-resistance with macrolides and are also used in food animals, this risk assessment was restricted to tylosin and tilmicosin. Consistent with CVM guidelines, a company or individual using the CVM framework should model only a single drug. The example assessment modeled tylosin and tilmicosin together, however, because of their close structural relationship. The risk assessment considered all label claim uses for both macrolides in the United States for poultry, swine, and beef cattle.

Because foodborne transmission of an antimicrobial resistance determinant (RzD; a genetic element that confers antimicrobial resistance) was considered the most likely hazard, it was the only route modeled (Figure 5). The microorganisms evaluated were *Campylobacter* spp. and *E. faecium*. Although differences in host range are known for *C. jejuni* and *C. coli*, the species were not separated. *Salmonella* is inherently resistant to macrolides; therefore, human salmonellosis is not treated with the drugs and was not considered in the risk assessment.

The guidance document was followed to define the hazard, which is illness: (1) caused by foodborne bacteria having an antibiotic resistance-determinant; (2) attributed to a specified animal-derived meat commodity; and (3) treated with a human-use drug of the same class. For the purposes of the risk assessment, the hazard was thus defined as human illness that is: (1) caused by macrolide-resistant *Campylobacter* spp. or *E. faecium*; (2) attributable to consumption of contaminated poultry, pork, or beef; and (3) treated with a human antibiotic of the macrolide class (FDA/CVM 2002). Risk was defined as the probability of the hazard combined with the consequence of treatment failure due to resistant *Campylobacter* spp. or *E. faecium*. A binomial event model was applied to estimate the annual risk for the U.S. population. Parameters were derived from industry drug use surveys, scientific literature, medical guidelines, and government documents. In all situations where there was a wide variation or uncertainty of data estimates, the most conservative (risk producing) estimates were used.

The FDA guideline treats the consequence assessment as a separate risk assessment, based on the drugs' importance to human medicine. Therefore, this method of combining the probability of an event with the consequences was a slight deviation from the guidance. In the study, the risk was defined and modeled as the yearly probability that an average individual in the U.S. population would be affected by the defined hazard and would experience an adverse therapeutic event (that is, poorer efficacy than usual as manifested by longer duration of diarrhea, progression to more severe disease, or unlikely mortality). A FoodNet review of 11275 human *Campylobacter* infections, showed that only 7 (0.006%) individuals died; less than 1% of cases were invasive (Kennedy and others 2000).

As noted, the example risk assessment is quantitative, as opposed to the qualitative type of assessment proposed by CVM. Data and resource constraints associated with a full-scale stochastic quantitative risk assessment led the CVM to recommend the simpler type of analysis, using high, medium, and low estimates for each of the three analyzed components—release-, exposure-, and consequence-assessment. However, CVM did not prohibit the quantitative approach. This example, however, uses a deterministic quantitative model which provides greater transparency regarding calculations and assumptions at each point in the chain of events. Additionally, a quantitative risk assessment can be revised as improved data estimates become available.

For human illness to occur as a result of antibiotic administration to a food animal, a number of events must occur. As generalized in Figure 5, the antibiotic must be administered to food animals. An increased prevalence of RzD must occur in the intestinal bacterial flora of the animals due to tylosin and tilmicosin administration. The resistant bacteria (*Campylobacter* spp. or *E. faecium* containing macrolide resistance genes) must leave the place of administration, for example, farm or feedlot. The RzD must move from the intestine in the treated animal to contaminate the carcass, rinse fluids, and/or neighboring carcasses during slaughter and processing operations and must survive processing, storage, and placement into the retail consumer sales environment. The meat product must then be mishandled, undercooked, or otherwise improperly prepared such that human infection or colonization can occur. For *Campylobacter* spp. the inoculating dose must be sufficient to cause the person to become ill, to seek medical treatment, and to be treated with a macrolide which would consequently be ineffective due to the RzD. In addition to being consistent with the CVM-defined hazard, the model provided an estimate of the probability that treatment would be ineffective (treatment failure), in terms of expected illness/per capita-year in the United States for which human macrolide treatment is presumed to fail or to be compromised by the presence of resistant bacteria due to administration of tylosin and tilmicosin to food animals.

This farm-to-patient risk assessment demonstrated that use of tylosin and tilmicosin in food animals presents a very low risk of human treatment failure, with an approximate annual probability of <1 in 10 million of treatment failure during human illness in the United States due to macrolide-resistant campylobacteriosis for all meat commodities combined. For poultry, the probability was slightly less than 1 in 14 million. For beef and pork, the probabilities were 1 in 53 million and 1 in 236 million, respectively. High *Campylobacter* spp. carcass contamination rates presumably drove the increased risk of treatment failure due to macrolide use in poultry. However, the estimated risk of 1 in 14 million was much less than fluoroquinolone-resistant *Campylobacter* spp. in chickens (1 in 30000), as reported in a CVM risk assessment (FDA/CVM 2001).

This model also indicated far less than one potential case per year of macrolide treatment failure from food-derived enterococcal infections in the United States (1 in 3 billion). This low result is due to the combined low level of macrolide susceptibility in *E. faecium* and the extremely low probability that foodborne enterococcal infections will occur in humans.

This example shows, using a rigorous quantitative model and conservative assumptions, that the foodborne risk of macrolide use in poultry and other livestock is estimated to be very low (<1 in 10 million). This analysis suggests that policies regarding antibiotic use in food animals should be developed on a case-by-case basis. Additionally, the potential benefits of antibiotic use, such as more uniform food animal quality, better evisceration, and reduced levels of pathogen (*Salmonella* spp., *Campylobacter* spp.) carcass contamination should also be considered.

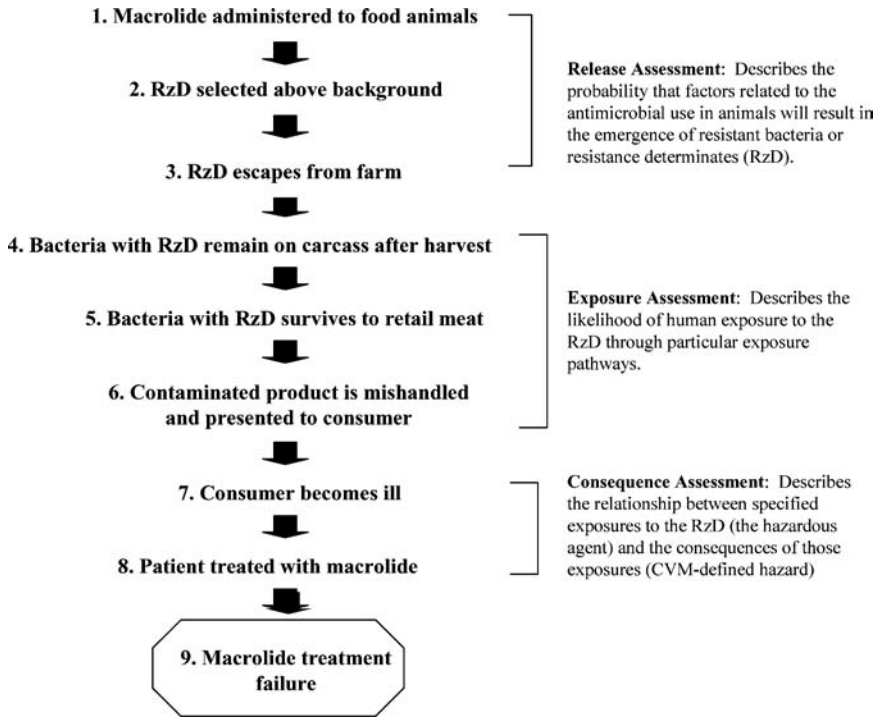

Figure 5—Chronology of treatment failure due to antimicrobial resistance. Reprinted with permission from the *Journal of Food Protection*. Copyright held by the International Association for Food Protection, Des Moines, Iowa, U.S.A. (Hurd HS, College of Veterinary Medicine, Iowa State University and others).

A review of recent risk management actions, such as elimination of antibiotic uses labeled for growth promotion in Europe (avoparcin, bacitracin, spiramycin, tylosin, and virginiamycin), has resulted in increased intestinal disease in animals and the concomitant use of more therapeutic antibiotics with a resultant increase in resistance (DANMAP 2004; WHO 2003). The discontinuation in the EU of use of antimicrobials for growth promotion has not been shown to have reduced the prevalence of certain antibiotic-resistant strains in human medicine; in fact, resistance increased among some pathogens, for example, tetracycline-resistant *Salmonella* Typhimurium, ampicillin-resistant *Salmonella* Typhimurium, tetracycline-resistant *C. jejuni*, erythromycin-resistant *C. jejuni*, virginiamycin-resistant *E. faecium*, tetracycline-resistant *E. faecium*, and ampicillin-resistant *E. coli*). Additionally, discontinuation of growth promotants was followed by increased therapeutic uses in some food animal production sectors. Further, the prevalence of resistant strains decreased for some antibiotics in some animals, but increased for other antibiotics and other bacteria in other animals. For example, while the total use of antibiotics in animals in Denmark decreased 30% between 1997 (before the ban) and 2004, there was a 41% increase between 1999 (after the ban) and 2004. During the 5-y period (1999 to 2004), resistance to tetracycline and ampicillin of *Salmonella* Typhimurium isolates from pigs increased. Resistance of *Salmonella* Typhimurium isolates from poultry increased from 0% in 1997 to 17% in 2004. Resistance of isolates from ill humans increased from 18% to 46% (DANMAP 2004).

A WHO review (WHO 2003) said, "It is probable, however, that termination of antimicrobial growth promoters had an indirect effect on resistance to tetracycline among *Salmonella* Typhimurium because of an increase in therapeutic tetracycline use in animals . . . . Increased tetracycline resistance among *Salmonella* is therefore not likely to result in ineffective treatment of *Salmonella* infections. Increased tetracycline resistance among *Salmonella* may result in additional human *Salmonella* infections, however, since persons who take tetracycline for other reasons are at an increased risk of becoming infected with tetracycline-resistant *Salmonella*." If the measure of success is reduced resistance in animals or humans, the ban in Denmark had mixed success. WHO said, "From a precautionary point of view, Denmark's program of antimicrobial growth promoter termination appears to have achieved its desired public health goal." The Danish experience is instructive for showing that thorough risk assessments should be used to guide selection of risk management actions so that unintended consequences are avoided or minimized.

Faced with concerns about the impact that the use of antibiotics in agriculture poses to public health, the FDA developed a set of guidelines, entitled "A Proposed Framework for Evaluating and Assuring the Human Safety of the Microbial Effects of Antimicrobial New Animal Drugs Intended for Use in Food-Producing Animals." The guidelines designate antibiotics into three classes: (1) drugs that are: [a] essential for treating a serious or life-threatening disease in humans (conditions of high morbidity or mortality) for which no satisfactory alternative therapy exists, [b] important for treating foodborne diseases in humans where resistance to alternative antimicrobial drugs may limit therapeutic options, or [c] a member of a class of drugs for which the mechanism of action and/or the nature of resistance induction is unique, and resistance to the antimicrobial is rare among human pathogen(s), and the drug holds potential for long-term therapy in human medicine; (2) of choice or important in treating a potentially serious disease, whether foodborne or otherwise, but for which satisfactory alternative therapy exists; and (3) which either have little or no use in human medicine, and are not the drug of first choice or a significant alternative for treating human

infections, including foodborne infections (FDA/CVM 1999). However, since it is impossible to predict the future for antibiotic discovery for substances for human use, the question arises as to whether analogs of current class 3 compounds could become class 1 drugs. The "Framework Document" was ultimately followed by FDA's issuance of its Guidance for Industry #152.

In 2006, the EU banned the remaining 4 nonhuman classes of antibiotics used in feed for growth promotion on the basis of the precautionary principle. This is a highly controversial and much debated concept originally developed for use in environmental protection but which is an element of human, animal, and plant health, as well as environmental risk management decision-making in the EU when scientific information is insufficient, inconclusive, or uncertain. In its strongest formulations, the precautionary principle can be interpreted as calling for absolute proof of safety before allowing new technologies to be adopted (Foster and others 2000). For example, the World Charter for Nature (WCN 1982) states "where potential adverse effects are not fully understood, the activities should not proceed." If interpreted literally, no new technology could meet this requirement. Pointing out the importance of mathematical models to help understand underlying mechanisms and guide policy responses, Smith and others (2005) stated that, given the intrinsic problem of knowability of the effects of agricultural antibiotic use on human health and the biological complexity of the problem, "precautionary decision making" is particularly suitable in this arena.

In the United States, precaution is embedded in the food safety system as an inherent part of relevant food safety statutes and regulations, as well as risk analysis policies and processes (risk assessment and risk management [FDA USDA 2000]). With regard to antimicrobial resistance, this inherent precaution has involved taking progressive action that included issuing draft industry guidance—the framework document—which introduced risk management, industry guidance pertaining to consideration of resistance in drug approvals, risk assessments, partnerships, and research (FDA USDA 2000).

Phillips and others (2004) conducted a critical review of published data to determine whether the use of antibiotics in food animals poses a risk to human health. They determined that the beneficial consequences of agricultural use of antimicrobials might very well outweigh the adverse effects. Moreover, they stated that the banning of any antibiotic usage in animals based on the precautionary principle in the absence of a full quantitative risk assessment is likely to be wasted at best and even harmful to animal and human health. For example, banning agricultural use of antibiotics might increase the pathogen load on the animals, which would increase the number of humans becoming ill, increasing the use of antibiotics in human medicine, and ultimately increasing the prevalence of antibiotic resistant pathogens and treatment failure.

The complexity of the antibiotic resistance issue precludes simple solutions. Resistance proclivity varies with the antimicrobial, bacterium, and usage patterns. Therefore, sweeping risk management measures that are proposed for a certain classification of use (nontherapeutic, growth promotion, and routine disease prevention, for example) can be draconian and without predictable results. Analysis must be carried out on a case-by-case basis, and driven by product specific, science-based risk assessments. The most effective way to address the complexity and totality of the farm-to-food-to-failure chain is to use a risk assessment approach. Conducting a risk assessment for a specific product use and tracking those bacteria that may become resistant as a result of that use would provide insight into what mitigation interventions would be most effective.

Given the impact of actions of the EU, EPA, and FDA and those of some corporations, incentives to develop new antibiotics for agricultural use have been severely diminished. Consequently, most large pharmaceutical and agrichemical companies are decreasing or abandoning their new antibiotic discovery efforts for agricultural use. While this may have some positive effect on public health, the effect is that there will be few or no new antibiotics for use in livestock or plants.

Data Gaps

Further research into mechanisms of action of antibiotics, food antimicrobial agents, sanitizers; microbial resistance to these agents; and genetic transfer of resistance determinants has implications for the medical, public health, veterinary, and food science and technology communities. A number of data gaps pertinent to specific sectors of the food system are outlined below.

Microbial ecology

- Identify environmental reservoirs of resistant microorganisms
- Elucidate the rate of transfer of resistance genes from bacteria in the environment to fecal flora of the human gastrointestinal tract
- Elucidate whether fungi in human mycotic infections have the same resistant genes as agriculturally significant fungi belonging to the same taxa

Microbial pathogenicity

- Determine the impact of antibiotic resistance on foodborne pathogen virulence
- Determine the impact of acid tolerance induction on foodborne pathogen dose response
- Correlate data from sentinel studies, including trends in susceptibility of key bacteria, on clinical outcomes of antibiotic use to microbiological endpoints
- Determine variability in avian flu isolates from poultry that are resistant to antiviral agents

Food production

- Clarify understanding of the mechanism for growth promotion effects of antimicrobials, to enable exploration of novel, effective, alternatives
- Conduct epidemiological and molecular level investigations to determine if antibiotic use in plant agriculture and aquaculture correlates with antibiotic resistance in human microflora, which would be particularly valuable in countries where antibiotic use on plants or in aquaculture is greater than in the United States
- Quantify on-farm selection for resistance, above the background, among zoonotic pathogens

Food manufacturing

- Using validated methods, determine the mechanisms of resistance and adaptation of microorganisms to food antimicrobial agents
- Investigate the bacterial stress hardening phenomena in actual food systems
- Elucidate the relationship between laboratory findings of resistance or adaptation to stress and food manufacturing microbial control practices
- Determine the stressing influences of foods and food processing environments, including biofilms, on resistance and virulence
- Enhance understanding of the genetic basis of antimicrobial resistance and adaptation to stresses
- Confirm that antibiotic-resistant microorganisms respond to interventions in a similar fashion as susceptible microorganisms

- Develop new, improved interventions to control foodborne pathogens based on optimized, efficient, economical, and integrated approaches that prevent resistance development and virulence enhancement, and assure product quality, processing efficiency, and control of resistant pathogens[18]
- Optimize the application of the hurdle concept by increasing knowledge of microbial regulatory processes under various environmental conditions, and exploring strategies that can induce activation of genes that sensitize microorganisms to subsequent stresses.

Conclusions

The United States has a complex and interdependent food production and manufacturing system that functions to meet the demands of the U.S. population and an active export market. Antimicrobials are important tools that are integral to food production and manufacturing. Beneficial antimicrobial applications are numerous, ranging from providing for high quality or good physical condition of crops, to good health of food animals entering the food chain, and maintaining sanitation during food processing.

Antibiotics are used to treat, prevent, and control disease among food animals and in some cases to improve feed utilization and, thus, growth rate. Administration of antibiotics to food animals is one aspect of an overall management system that is a critical component in securing the health and welfare of the animals as well as the safety of the products derived from them. Further, several nonantibiotic antimicrobials, including disinfectants and sanitizers are used to disinfect or sanitize animal production premises, transport equipment, carcasses, and slaughter facility equipment. These substances are an important part of pathogen reduction strategies. Sanitizing and decontaminating agents are used to control microorganisms on fresh produce. Several different types of antimicrobial agents are used in food manufacturing to either clean food manufacturing environments or ensure food quality and safety. In addressing quality and safety, traditional and naturally occurring food antimicrobials are increasingly applied as multiple, synergistic hurdles to inactivate or inhibit growth of spoilage and pathogenic microorganisms. The use of multiple hurdles in food manufacturing is likely to combat resistance to singular food safety interventions.

Although the total amount of antimicrobials used in human medicine and agriculture is not precisely known, both sectors use appreciable quantities. Estimates of use are influenced by data gaps and inaccuracies. Estimates of the total amount of antibiotics produced annually during the 1970s, 1980s, and 1990s range from 14.0 to 22.7 million kg (31 to 50 million pounds). Estimates of the amount of antibiotics used in production agriculture range from 18.4 to 30 million pounds. Quantity of use, however, does not necessarily correspond with efficacy in antibiotic use in humans, animals, or plants.

The availability of antibiotics to treat infectious diseases has radically improved human and animal well-being. Paradoxically, this very success threatens their future utility. Both the prudent and inappropriate use of antibiotics in human medicine, veterinary medicine, and animal husbandry create selective pressure that favors the emergence of antibiotic resistant microbes. Coupled with specific genetic resistance mechanisms, the selective pressure of antimicrobials may result in foodborne bacteria that are resistant to antimicrobials. Antibiotic resistance among foodborne pathogens may create an increased burden to human health in different ways: (1) resistant pathogens contaminating food animals have the potential to reach humans; (2) human use of antibiotics may increase the risk of acquiring an infection with an antimicrobial resistant pathogen; (3) human infection with a resistant microbe may limit illness treatment options (in the uncommon instances of foodborne illness in which antibiotic use is warranted); and (4) antibiotic-resistant foodborne pathogens may develop increased virulence. Of these potential impacts, prior exposure of humans to antibiotics is the greatest risk factor for acquiring an infection with antibiotic-resistant bacteria. The preponderance of evidence strongly supports the suggestion that antibiotic resistance results in a larger number of human infections by increasing the risk of infection in people who have had prior antibiotic exposure.

Antibiotic-resistant foodborne pathogens are a subset of foodborne pathogens, any of which may cause illness. Antibiotic-resistant intestinal bacteria may be present in food animals, regardless of exposure of the animals to an antibiotic. The types of bacteria, their resistance profiles, and prevalence vary from animal to animal and species to species. In spite of the best efforts to prevent or eliminate them, some antibiotic-resistant bacteria contaminate carcasses, as do antibiotic susceptible bacteria. Interventions that effectively reduce the prevalence of foodborne pathogens also reduce the prevalence of those that are resistant to antibiotics.

There are a variety of resistance mechanisms and genes that complicate the antibiotic resistance issue. Commensals, such as nonpathogenic *E. coli* and *Enterococcus* spp., may serve as reservoirs of potential antimicrobial resistance genes in the environment from which resistance may be transferred to other commensals or pathogenic bacteria. However, of singular interest are those antibiotic-resistant intestinal bacteria, such as *Salmonella* and *Campylobacter* that can contaminate foods during slaughter or processing and result in human illness. The key points of influence that food scientists have in preventing the spread of antibiotic-resistant and sensitive pathogenic microorganisms in foods are preventing them from entering the food supply, and if present, inactivating them or preventing their growth.

Selective pressure for the development of antimicrobial resistance occurs within all uses of antimicrobials, including use in the food system from production to processing. Resistance among some foodborne bacterial pathogens has increased during the past 15 to 25 years. Increases in resistance have generated heated debate about the appropriate use of antibiotics in agriculture, particularly in food animal production. Although the people involved in the various stages of the food system can influence dissemination of foodborne pathogens, including those resistant to antibiotics, through various intervention strategies, they control neither the development of antibiotic resistance nor human antibiotic use patterns. Given the different resistance mechanisms, conditions selecting for resistance, and dissemination patterns of resistant microorganisms, a single approach to solving the resistance issue is not possible.

Various factors complicate our ability to fully understand the transfer of resistant bacteria through the food chain to human illness causation. These factors include resistance genes unique to the various foodborne pathogens; animal production and distribution prior to slaughter; processing practices; retail food preparation, distribution, and storage; consumer food preparation practices; varying susceptibility to pathogens among different subpopulations; and varying medical practices and treatment options.

[18] Additional studies of this type are necessary to examine the hypothesis that stress-resistant or adapted pathogens may be of more concern in food safety than their sensitive counterparts. Results could be useful in proving or disproving hypotheses such as the suggestion that pathogen resistance to food-related stressors may have played a role in the new involvement in foodborne illness of traditionally low risk foods, such as fruit juices, fermented meats, fresh produce, and dried products.

The extent to which antibiotic use in food animals produces clinically important antibiotic resistant infections in humans is unknown. Contributing to this problem is the inability to obtain quantitative data about the magnitude of antibiotic use in animal husbandry, subsequent resistance, and impact on human health. Additionally, the economic impact of antibiotic resistance is difficult to assess, as are potential affects on trade.

To address the complexity of resistance selection, transfer through the food chain, and human health consequences, qualitative and quantitative risk assessments are now being applied. For many antibiotics—such as tylosin, tilmicosin, and virginiamycin used in food animals and for which a risk assessment has been conducted—estimated risk to human health is small. Fluoroquinolone use to treat poultry disease through water, however, was deemed by the FDA as an unacceptable risk to humans and its approval was withdrawn. The FDA/CVM now requires new animal drug sponsors to satisfy microbial food safety criteria for antibiotic products by submitting evidence outlined in Guidance 152 that appropriate use conditions are ensured.

Risk management strategies to minimize and contain antibiotic-resistant foodborne bacteria are in place all along the food chain, but can be improved. The strategies that have been implemented include use of various antibiotic alternatives, implementation of judicious or prudent antibiotic use guidelines, and implementation of national resistance monitoring programs.

Although there are concerns with antibiotics entering the animal production environment through manure or other waste streams, more information is needed to better understand the situation to implement effective control strategies. Very little is known about the exposure routes of antimicrobials in the environment and the fate of antimicrobials within ecosystems; environmental impacts are not completely understood. Current evidence suggests that it is not likely that antimicrobials in manure will pose any direct risk to soil microbiota. However, it is not yet possible to exclude other indirect effects on soil microbiota and ecosystems that are driven by changes in the microbial community from the presence of antibiotics. Environmental research is in its infancy, currently able to simply identify whether a hazard exists and is not yet able to measure impact.

Although bacteria may be exposed to an antibiotic for an intended period of time, on the farm or in humans, bacterial exposure to food antimicrobials (for example, sanitizers) generally occurs only once. The prevalence and mechanism of resistance among most food-use antimicrobial compounds is often unknown. When it occurs, resistance to food antimicrobials is of little practical relevance to the food industry because the antimicrobial concentrations used in food manufacturing are well above the low-level bacterial resistance (a comparatively low MIC). However, the ability of some sanitizers and disinfectants to induce MDR-pumps, which also confer antibiotic resistance, is of some concern.

The impact on human health of bacterial pathogen resistance to food antimicrobials is not fully understood. Although some studies have suggested that in certain situations (sublethal use, overuse, biofilms, and cross-resistance mechanisms, for example) the potential for negative impact on public health exists, resistance to food antimicrobials is not considered a major public health concern because the resistance mechanisms are often temporary adaptations. To date, the use in foods of chemical and biological antimicrobials and physical preservation systems has been remarkably successful in providing safe foods and has not been compromised by the occurrence of resistant microorganisms.

Monitoring and surveillance of antibiotic resistance in plant production agriculture is not done on a regular basis, and the effects on the microflora of applicators and transient visitors, including workers in treated fields and orchards, have not yet been investigated. At present there is little evidence of an impact on human health of use of antibiotics in plant production. Similarly, ingestion of antibiotic-resistant bacteria from aquaculture and contact with animals, including pets, does not appear to comprise a significant threat to human health.

NARMS and FoodNet surveillance data are now beginning to reveal resistance trends. NARMS resistance trends are not consistently in one direction. Trends reported by other surveillance programs during the past 20 to 25 years reveal increasing resistance, while other sources reveal decreasing resistance trends particularly in the last 6 to 7 years.

It is difficult to correlate antibiotic resistance among foodborne pathogens with particular types of antibiotic use (for example, therapeutic growth promotion) on the farm. Increased incidence of illness within a herd or flock, and concomitant therapeutic use of antibiotics in any given year may or may not result in increased use of antibiotics potentially selecting for resistant microorganisms. Therefore, it is difficult to compare year-to-year resistance trend data without correlating the data with disease prevalence and corresponding changes in annual use of a specific antibiotic or class of antibiotics.

FoodNet trends of foodborne illness show a decline in salmonellosis and a decline in campylobacteriosis to levels approaching the national health objective targets for the year 2010 (CDC 2004b). The declines may be due in part to HACCP implementation, pathogen reduction actions in food slaughter and manufacturing facilities, and other intervention modalities.

The history of the epidemiology of *Salmonella* shows that clones, including MDR-clones, spread worldwide, and then lost predominance. Some clones of *Salmonella* Typhimurium DT104, which possess a penta-resistance gene cassette, have spread widely and resulted in foodborne disease outbreaks. It appears that the prevalence of *Salmonella* Typhimurium DT104 and/or the penta-resistant *Salmonella* Typhimurium may have peaked in 1996, and declined since then.

Regulatory targeting of specific antibiotic-resistant foodborne pathogens may not be the most successful or cost effective means to reduce overall foodborne illness. A HACCP approach applied throughout the food chain is considered the most effective measure to controlling foodborne pathogens and thereby reducing foodborne illnesses. Most interventions, critical control points to kill or reduce foodborne pathogens, for example, are equally effective in controlling microbes regardless of their resistance to antibiotics. Thus, applying interventions to control foodborne pathogens in general, rather than focusing on antibiotic-resistant strains specifically, would have the greatest impact in reducing overall foodborne illnesses.

There are limited new veterinary drugs in the pipeline. Of drugs under development, many of them are targeted for noninfectious diseases. Although alternatives to antibiotics have been explored, none can replace those used for therapeutic purposes. Thus, maintaining the continued efficacy of currently available antibiotics is critical.

Specific recommendations

Antibiotic resistance among microorganisms, commensal and pathogenic alike, is a concern for food safety worldwide. Resistance can be controlled or mitigated, however, in a number of ways. Those who control or administer antibiotic use in human medicine, veterinary medicine, and production agriculture can have the greatest impact in controlling resistance. In human medicine, practice of appropriate therapy and use of improved patient diagnostics and treatments minimize resistance selection. In veterinary medicine and production agriculture implementation of various management strategies (such as responsible use guidelines, quality assurance programs, and antibiotic

alternatives), coupled with government regulations, should decrease opportunities for the selection of antibiotic-resistant microorganisms. Despite the significant role that many people have in controlling antibiotic resistance and its potential impacts, the IFT Expert Panel concluded that the following areas warrant attention or investigation.

- Increase attention to the public health benefits, as well as risks, of losing the efficacy of existing and future antimicrobials.
- Determine the public health impact of antimicrobial resistance on the basis of risk assessment, and consider resistance on the basis of an individual microorganism exposed to a specific agent under a specific condition of use.
- Guide risk management strategies by the results of risk assessments.
- Always practice prudent use of antimicrobials to limit resistance selection and to maintain maximal benefit of antimicrobials in the future.
- Expand development of prudent use guidelines to include all antibiotic uses. Prudent use does not necessarily correlate with reduced use; an unknown risk of maintaining use may be less than an equally unknown risk of reducing use.
- Modify prudent use guidelines as new scientific evidence on antimicrobial resistance becomes available.
- Develop, validate, and implement prudent use guidelines for bactericidal food antimicrobial agents and sanitizers.
- Conduct more research to identify effective alternatives to antibiotics.
- Implement surveillance programs and food attribution models as means for measuring the effectiveness of the food industry's microbiological interventions.
- Determine and evaluate the relationship between use of specific antibiotics in food animal husbandry to resistance selection rates among major foodborne bacteria at slaughter on farms where antibiotics are used and farms where antibiotics are not used.
- Initiate characterization of resistance to food antimicrobial agents and sanitizers.
- Advance understanding of the mechanisms of resistance to food antimicrobial agents and sanitizers.
- Improve the ability of scientists to predict the potential for cross-resistance with antibiotics through increased focus on determining and understanding mechanisms of resistance.
- Aid in elucidating reasons that some combinations and sequences of antimicrobial interventions result in synergistic "multiple hurdle" effects while others cause stress-hardening or adaptation through increased knowledge of mechanisms of resistance.
- Implement further study to confirm that current data indicate that microbial interventions are equally effective for antimicrobial susceptible and resistant microorganisms.

References

[AAM] American Academy of Microbiology. Antimicrobial resistance: an ecological perspective. Washington, D.C.: AAM. Available from: http://www.asm.org/Academy/index.asp?bid=2167. Accessed: June 24, 2002.

Aase B, Sundheim G, Langsrud S, Rorvik LM. 2000. Occurrence of and a possible mechanism for resistance to a quaternary ammonium compound in *Listeria monocytogenes*. Intl J Food Microbiol 62:57–63.

Abee T, Wouters JA. 1999. Microbial stress response in minimal processing. Intl J Food Microbiol 50:65–91.

Abouzeed YM, Hariharan H, Poppe C, Kibenge FS. 2000. Characterization of Salmonella isolates from beef cattle, broiler chickens and human sources on Prince Edward Island. Comp Immunol Microbiol Infect Dis 23:253–66.

Acar J, Rostel B. 2001. Antimicrobial resistance: an overview. Rev Sci Tech 20:797–810.

Adamczyk M, Jagura-Burdzy G. 2003. Spread and survival of promiscuous IncP-1 plasmids. Acta Biochim Pol 50:425–53.

Agerso Y, Sengelov G, Jensen LB. 2004. Development of a rapid method for direct detection of tet(M) genes in soil from Danish farmland. Environ Intl 30:117–22.

Agrios GN. 2005. lant pathology. 5th ed. Amsterdam: Elsevier Academic Press. 922 p.

[AHI] Animal Health Institute. 2000. Survey indicates most antibiotics used in animals are used for treating and preventing disease. Press release., Washington D.C.: AHI.

[AHI] Animal Health Institute. 2002. Survey shows decline in antibiotic use in animals. News release. Washington, D.C.: AHI.

[AHI] Animal Health Institute. 2005. Antibiotic use in animals rises in 2004. News release. Washington, D.C.: AHI.

Ahmer BM, Tran M, Heffron F. 1999. The virulence plasmid of *Salmonella typhimurium* is self-transmissible. J Bacteriol 181(4):1364–8.

Aiello AE, Marshall B, Levy SB, Della-Latta P, Lin SX, Larson E. 2005. Antibacterial cleaning products and drug resistance. Emerg Infec Dis [serial on the internet]. 11(10). Available from: http://www.cdc.gov/ncidod/EID/vol11no10/04-1276.htm.

Alekshun MN, Levy SB. 1999. The mar regulon: multiple resistance to antibiotics and other toxic chemicals. Trends Microbiol 7:410–3.

Altekruse SF, Elvinger F, DebRoy C, Pierson FW, Eifert JD, Sriranganathan N. 2002. Pathogenic and fecal *Escherichia coli* strains from turkeys in a commercial operation. Avian Dis 46:562–9.

Anderson KB, Tan JS, File TM Jr., DiPersio JR, Willey BM, Low DE. 2003. Emergence of levofloxacin-resistant pneumococci in immunocompromised adults after therapy for community-acquired pneumonia. Clin Infect Dis 37:376–81.

Andrup L, Andersen K. 1999. A comparison of the kinetics of plasmid transfer in the conjugation systems encoded by the F plasmid from *Escherichia coli* and plasmid pCF10 from *Enterococcus faecalis*. Microbiol 145:2001–9.

Angulo FJ, Johnson KR, Tauxe RV, Cohen ML. 2000. Origins and consequences of antimicrobial-resistant nontyphoidal *Salmonella*: implications for the use of fluoroquinolones in food animals. Microb Drug Resist 6:77–83.

Anonymous. 2002. Outbreak of multidrug-resistant Salmonella newport—United States, Jan–Apr. Morb Mortal Wkly Rep 51:545–8.

Anonymous. 2006a. Feed additive compendium. Miller Publishing Company. Available from: http://www.feedstuffs.com/ME2/dirsect.asp?sid=9E937630D3A44C80B1F1AD3D6080D22F&nm=Other+Products

Anonymous. 2006b. Direct-fed microbial, enzyme and forage additive compendium. Miller Publishing Company. Available from: http://www.feedstuffs.com/ME2/dirsect.asp?sid=9E937630D3A44C80B1F1AD3D6080D22F&nm=Other+Products

Anthony KG, Klimke WA, Manchak J, Frost LS. 1999. Comparison of proteins involved in pilus synthesis and mating pair stabilization from the related plasmids F and R100-1: insights into the mechanism of conjugations. J Bacteriol 181(17):5149–59.

Aoki T. 2005. personal communication.

Aoki T, Kitao T. 1985. Detection of transferable R plasmids in strains of the fish pathogenic bacterium, *Pasteurella piscicida*. J Fish Dis 8:345–50.

APUA. 2006. Practitioner guidelines. Alliance for the Prudent Use of Antibiotics. Available from: http://www.tufts.edu/med/apua/Practitioners/healthcare.html). Accessed: June 24, 2002.

Arakawa Y, Murakami M, Suzuki K, Ito H. 1995. A novel integron-like element carrying the metallo-β-lactamase gene bla_{IMP}. Antimicrob Agents Chemother 39:1612–5.

Arcangioli MA, Leroy-Setrin S, Martel JL, Chaslus-Dancla E. 1999. A new chloramphenicol and florfenicol resistance gene flanked by two integron structures in *Salmonella* typhimurium DT104. FEMS Microbiol Lett 174:327–32.

Archer DL. 1996. Preservation microbiology and safety: evidence that stress enhances virulence and triggers adaptive mutations. Trends Food Sci Technol 7:91–5.

Aubry-Damon H, Grenet K, Sall-Ndiaye P, Che D, Cordeiro E, Bougnoux ME, Rigaud E, Le Strat Y, Lemanissier V, Armand-Lefevre L, Delzescaux D, Desenclos JC, Lienard M, Andremont A. 2004. Antimicrobial resistance in commensal flora of pig farmers. Emerg Infect Dis 10:873–9.

[AVA] Australian Veterinary Association. 1999. Code of practice for the use of antimicrobial drugs in veterinary practice. Artarmon, New South Wales, Australia: AVA.

Avorn J, Solomon DH. 2000. Cultural and economic factors that (mis)shape antibiotic use: the nonpharmacologic basis of therapeutics. Ann Intern Med 133:128–35.

Bacciu DG, Falchi A, Spazziani A, Bossi L, Marogna G, Leori GS, Rubino S, Uzzau S. 2004. Transposition of the heat-stable toxin AstA gene into a Gifsy-2-related prophage of *Salmonella enterica* serovar Abortusovis. J Bacteriol 186(14):4568–74.

Bacon RT, Ransom JR, Sofos JN, Kendall PA, Belk KE, Smith GC. 2003a. Thermal inactivation of susceptible and multiantimicrobial-resistant salmonella strains grown in the absence or presence of glucose. Appl Environ Microbiol 69:4123–8.

Bacon RT, Sofos JN, Kendall PA, Belk KE, Smith GC. 2003b. Comparative analysis of acid resistance between susceptible and multi-antimicrobial-resistant Salmonella strains cultured under stationary-phase acid tolerance-inducing and noninducing conditions. J Food Protect 66:732–40.

Baer JT, Vugia DJ, Reingold AL, Aragon T, Angulo FJ, Bradford WZ. 1999. HIV infection as a risk factor for shigellosis. Emerg Infect Dis 5:820–3.

Baggesen DL, Aarestrup FM. 1998. Characterization of recently emerged multiple antibiotic-resistant *Salmonella enterica* serovar typhimurium DT104 and other multiresistant phage types from Danish pig herds. Vet Rec 143:95–7.

Baguer AJ, Jensen J, Krogh PH. 2000. Effects of the antibiotics oxytetracycline and tylosin on soil fauna. Chemosphere 40:751–7.

Bargiota E, Rico-Muñoz E, Davidson PM. 1987. Lethal effect of methyl and propyl parabens as related to *Staphylococcus aureus* lipid composition. Intl J Food Microbiol 4:257-66.

Barlow RS, Pemberton JM, Desmarchelier PM, Gobius KS. 2004. Isolation and characterization of integron-containing bacteria without antibiotic selection. Antimicrob Agents Chemother 48(3):838–42.

Barmpalia IM, Geornaras I, Belk KE, Scanga JA, Kendall PA, Smith GC, Sofos JN. 2004. Control of *Listeria monocytogenes* on frankfurters with antimicrobials in the formulation and by dipping in organic acid solutions. J Food Protect 67:2456–64.

Barmpalia IM, Koutsoumanis KP, Geornaras I, Belk KE, Scanga JA, Kendall PA, Smith GC, Sofos JN. 2005. Effect of antimicrobials as ingredients of pork bologna for *Listeria monocytogenes* control during storage at 4 or 10 °C. Food Microbiol 22:205–11.

Barrow PA. 2001. The use of bacteriophages for treatment and prevention of bacterial disease in animals and animal models of human infection. J Chem Technol Biotechnol 76:677–82.

Bartlett J. 2000. Treatment of community-acquired pneumonia. Chemother 46(Suppl 1):24–31.

Barza M, Travers K. 2002. Excess infections due to antimicrobial resistance: the "attributable fraction." Clin Infect Dis 34(Suppl 3):S126–30.
Bass L, Liebert CA, Lee MD, White DG, Summers AO, Thayer SG, Maurer JJ. 1999. The incidence and characterization of integrons, genetic elements associated with multiple drug resistance, in avian *Escherichia coli*. Antimicrob Agents Chemother 43:2925–9.
Bates J, Jordens JZ, Griffiths DT. 1994. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J Antimicrob Chemother 34:507–14.
Bauer F, Hertel C, Hammes WP. 1999. Transformation of *Escherichia coli* in foodstuffs. Syst Appl Microbiol 22:161–8.
Bauernfeind A, Stemplinger I, Jungwirth R, Mangold P, Amann S, Akalin E, Ang O, Bal C, Casellas JM. 1996. Characterization of beta-lactamase gene blaPER-2, which encodes an extended-spectrum class A beta-lactamase. Antimicrob Agents Chemother 40:616–20.
Bedie GK, Samelis J, Sofos JN, Belk KE, Scanga JA, Smith GC. 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* post-processing contamination on frankfurters stored at 4 °C in vacuum packages. J Food Protect 64:1949–55.
Bennett PM, Livesey CT, Nathwani D, Reeves DS, Saunders JR, Wise R. 2004. An assessment of the risks associated with the use of antibiotic resistance genes in genetically modified plants: report of the Working Party of the British Society for Antimicrobial Chemotherapy. J Antimicrob Chemother 53:418–31.
Benson CE, Munro DS, Rankin S. 1997. Salmonella typhimurium DT104 in the northeast USA. Vet Rec 141(19):503–4.
Berg DE, Berg CM, Sasakawa C. 1984. Bacterial transposon Tn*5*: evolutionary inferences. Mol Biol Evol 1:411–22.
Berg T, Firth N, Apisiridej S, Hettiaratchi A, Leelaporn A, Skurray RA. 1998. Complete nucleotide sequence of pSK41: evolution of staphylococcal conjugative multiresistance plasmids. J Bacteriol 180:4350–9.
Berge AC, Adaska JM, Sischo WM. 2004. Use of antibiotic susceptibility patterns and pulsed-field gel electrophoresis to compare historic and contemporary isolates of multi-drug-resistant *Salmonella enterica* subsp. enterica serovar Newport. Appl Environ Microbiol 70:318–23.
Bertolatti D, Munyard SJ, Grubb WB, Binns CW. 2001. Thermal inactivation of antimicrobial-resistant Gram-positive cocci in chicken meat: D and Z value determinations. Intl J Environ Health Res 11:257–66.
Besser TE, Goldoft M, Pritchett LC, Khakhria R, Hancock DD, Rice DH, Gay JM, Johnson W, Gay CC. 2000. Multiresistant *Salmonella* Typhimurium104 infections of humans and domestic animals in the Pacific Northwest of the United States. Epidemiol Infect 124:193–200.
Beuchat LR, Ryu JH. 1997. Produce handling and processing practices. Emerg Infect Dis 3:459–65.
Bhargava HN, Leonard PA. 1996. Triclosan: applications and safety. Am J Infect Control 24:209–18.
Bhattacharya SK, Sarkar K, Balakrish Nair G, Faruque AS, Sack DA. 2003. Multidrug-resistant *Shigella dysenteriae* type 1 in south Asia. Lancet Infect Dis 3:755.
Bills S, Restaino L, Lenovich LM. 1982. Growth response of an osmotolerant sorbate-resistant yeast, *Saccharomyces rouxii* at different sucrose and sorbate levels. J Food Protect 45:1120–4.
Bodnaruk PW, Krakar PJ, Tompkin RB. 2001. Absence of high-level vancomycin resistance in enterococci isolated from meat-processing facilities. Emerg Infect Dis 7:1030–1.
Boerlin P, Eugster S, Gaschen F, Straub R, Schawalder P. 2001. Transmission of opportunistic pathogens in a veterinary teaching hospital. Vet Microbiol 82:347–59.
Bolton LF, Kelley LC, Lee MD, Fedorka-Cray PJ, Maurer JJ. 1999. Detection of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J Clin Microbiol 37:1348–51.
Bonnet M, Montville TJ. 2005. Acid-tolerant *Listeria monocytogenes* persist in a model food system fermented with nisin-producing bacteria. Lett Appl Microbiol 40:237–42.
Bopp CA, Greene KD, Downes FP, Sowers EG, Wells JG, Wachsmuth IK. 1987. Unusual verotoxin-producing *Escherichia coli* associated with hemorrhagic colitis. J Clin Microbiol 25:1486–9.
Bourn D, Prescott J. 2002. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. Crit Rev Food Sci Nutr 42:1–34.
Bower CK, Daeschel MA. 1999. Resistance responses of microorganisms in food environments. Intl J Food Microbiol 50:33–44.
Boyd D, Peters GA, Cloeckaert A, Boumedine KS, Chaslus-Dancla E, Imberechts H, Mulvey MR. 2001. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar TyphimuriumDT104 and its identification in phage type DT120 and serovar Agona. J Bacteriol 183:5725–32.
Bradford PA, Yang Y, Sahm D, Grope I, Gardovska D, Storch G. 1998. CTX-M-5, a novel cefotaxime-hydrolyzing beta-lactamase from an outbreak of *Salmonella* typhimurium in Latvia. Antimicrob Agents Chemother 42:1980–4.
Bradley CR, Fraise AP. 1996. Heat and chemical resistance of enterococci. J Hosp Infect 34:191–6.
Briggs CE, Fratamico PM. 1999. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob Agents Chemother 43:846–9.
Brul S, Coote P. 1999. Preservative agents in foods. Mode of action and microbial resistance mechanisms. Intl J Food Microbiol 50:1–17.
Buck JS, Walcott RR, Beuchat LR. 2003. Recent trends in microbiological safety of fruits and vegetables. Available from: http://www.plantmanagementnetwork.org/php/default.asp.
Burman LG. 1975. Amplification of sex repressor function of one fi- + R-factor during anaerobic growth of *Escherichia coli*. J Bacteriol 123:265–71.
Burnham JA, Kendall PA, Sofos JN. 2001. Ascorbic acid enhances destruction of *Escherichia coli* O157:H7 during home-type drying of apple slices. J Food Protect 64:1244–8.
Bush K, Sykes RB. 1984. Interaction of β-lactam antibiotics with β-lactamases as a cause for resistance. In: Bryan LE, ed. Antimicrobial drug resistance. New York: Academic Press, Inc.p 1–31.
Butler CA, Gotschlich EC. 1991. High-frequency mobilization of broad-host range plasmids into *Neisseria gonorrhoeae* requires methylation in the donor. J Bacteriol 173:5793–9.
[BVA] British Veterinary Association. 1998. General guidelines on the use of antimicrobials. Vet Rec 143(20):565–6.
[CAC] Codex Alimentarius Commission. 2005. Report of the 28th Session of the Codex Alimentarius Commission. Joint FAO/WHO Food Standards Programme. Jul 4–9. Rome, Italy: CAC. Available: www.codexalimentarius.net.
Calicioglu M, Sofos JN, Samelis J, Kendall PA, Smith GC. 2002a. Inactivation of acid-adapted and nonadapted *Escherichia coli* O157:H7 during drying and storage of beef jerky treated with different marinades. J Food Protect 65:1394–405.
Calicioglu M, Sofos JN, Samelis J, Kendall PA, Smith GC. 2002b. Destruction of acid- and non-adapted *Listeria monocytogenes* during drying and storage of beef jerky. Food Microbiol 19:545–59.
Calicioglu M, Sofos JN, Kendall PA. 2003a. Fate of acid-adapted and non-adapted *Escherichia coli* O157:H7 inoculated post-drying on beef jerky treated with marinades before drying. Food Microbiol 20:169–77.
Calicioglu M, Sofos JN, Kendall PA, Smith GC. 2003b. Effects of acid adaptation and modified marinades on survival of postdrying *Salmonella* contamination on beef jerky during storage. J Food Protect 66:396–402.
Calicioglu M, Sofos JN, Kendall PA. 2003c. Influence of marinades on survival during storage of acid-adapted and nonadapted *Listeria monocytogenes* inoculated post-processing on beef jerky. Intl J Food Microbiol 86:283–92.
Calicioglu M, Sofos JN, Samelis J, Kendall PA, Smith GC. 2003d. Effect of acid adaptation on inactivation of *Salmonella* during drying and storage of beef jerky treated with marinades. Intl J Food Microbiol 89:51–65.
Cagri A, Ustunol Z, Ryser ET. 2004. Antimicrobial edible films and coatings. J Food Protect 67:833–48.
Call DR, Bakko MK, Krug MJ, Roberts MC. 2003. Identifying antimicrobial resistance genes with DNA microarrays. Antimicrob Agents Chemother 47:3290–5.
Camacho EM, Casadesus J. 2002. Conjugal transfer of the virulence plasmid of *Salmonella enterica* is regulated by the leucine-responsive regulatory protein and DNA adenine methylations. Mol Microbiol 44:1589–98.
Camus AC. 2001. Pathobiology of *Streptococcus iniae* infections in cultured tilapia. Louisiana State University, Baton: Rouge.
Canchaya C, Foumous G, Brussow H. 2003. The impact of prophages on bacterial chromosomes. Molec Microbiol 53(1):9–18.
Carlberg J, Van Olst JC, Massengill M. 2000. Hybrid striped bass: an important fish in U.S. aquaculture. Aquaculture Mag 26:1–5.
Carlotti DN, Guaguere E, Pin D, Jasmin P, Thomas E, Guiral V. 1999. Therapy of difficult cases of canine pyoderma with marbofloxacin: a report of 39 dogs. J Small Anim Pract 40:265–70.
Cassell GH. 1997. Emergent antibiotic resistance: health risks and economic impact. FEMS Immunol Med Microbiol 18:271–4.
[CDC] Centers for Disease Control and Prevention. 2001a. Outbreaks of multidrug-resistant *Salmonella* Typhimurium associated with veterinary facilities—Idaho, Minnesota, and Washington, 1999. CDC Morb Mortal Wkly Rpt 50:701–4.
[CDC] Centers for Disease Control and Prevention. 2001b. A public health action plan to combat antimicrobial resistance. Part I. Domestic issues. Atlanta, GA. Available from: http://www.cdc.gov/drugresistance/actionplan/index.htm.
[CDC] Centers for Disease Control and Prevention. 2003. Preliminary FoodNet data on the incidence of foodborne illnesses—selected sites, United States, 2002. CDC. Morb Mortal Wkly Rpt 52:340–3.
[CDC]. 2004a. Campaign to prevent antimicrobial resistance in healthcare settings. Atlanta, GA. CDC. Available from: http://www.cdc.gov/drugresistance/healthcare/patients.htm.
[CDC] Centers for Disease Control and Prevention. 2004b. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-selected sites, United States, 2003. CDC. Morb Mortal Wkly Rpt. 53:338–43.
Cefai C, Ashurst S, Owens C. 1994. Human carriage of methicillin-resistant *Staphylococcus aureus* linked with pet dog. Lancet 344(8921):539–40.
Celum CL, Chaisson RE, Rutherford GW, Barnhart JL, Echenberg DF. 1987. Incidence of salmonellosis in patients with AIDS. J Infect Dis 156:998–1002.
Chapman JS. 1998. Characterizing bacterial resistance to preservatives and disinfectants. Intl Biodeter Biodegrad 41:241-5.
Chaslus-Dancla E, Lafont JP. 1985. IncH plasmids in *Escherichia coli* strains isolated from broiler chicken carcasses. Appl Environ Microbiol 49(4):1016–8.
Chattoraj DK. 2000. Control of plasmid DNA replication by iterons: no longer paradoxical. Mol Microbiol 37(3):467–76.
Chatzipanagiotou S, Papavasileiou E, Lakumenta A, Makri A, Nicolaou C, Chantzis K, Manganas S, Legakis NI. 2002. Antimicrobial susceptibility patterns of *Campylobacter jejuni* strains isolated from hospitalized children in Athens, Greece. J Antimicrob Chemother 49:803–5.
Chee-Sanford JC, Aminov RI, Krapac IJ, Garrigues-Jeanjean N, Mackie RI. 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. Appl Environ Microbiol 67:1494–502.
Chen S, Zhao S, White DG, Schroeder CM, Lu R, Yang H, McDermott PF, Ayers S, Meng J. 2004. Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. App Environ Microbiol 70(1):1–7.
Chibani-Chennoufi S, Bruttin A, Dillmann ML, Brussow H. 2004. Phage-host interaction: an ecological perspective. J Bacteriol 186:3677–86.
Chipley JR. 1993. Sodium benzoate and benzoic acid. In: Davidson PM, Branen AL, eds. Antimicrobials in foods. 2nd ed. New York: Marcel Dekker. p 11–48.
Chu C, Chiu CH, Wu WY, Chu CH, Liu TP, Ou JT. 2001. Large drug resistance virulence plasmids of clinical isolates of *Salmonella enterica* serovar Choleraesuis. Antimicrob Agents Chemother 45:2299–303.
Chung WO, Werckenthin C, Schwarz S, Roberts MC. 1999a. Host range of the *ermF* rRNA methylase gene in bacteria of human and animal origin. J Antimicrob Chemother 43:5–14.
Chung WO, Young K, Leng Z, Roberts MC. 1999b. Mobile elements carrying *ermF* and *tetQ* in gram-positive and gram-negative bacteria. J Antimicrob Chemother 44:329–35.
Clark NC, Olsvik O, Swenson JM, Spiegel CA, Tenover FC. 1999. Detection of a streptomycin/spectinomycin adenylyltransferase gene (*aadA*) in *Enterococcus faecalis*. Antimicrob Agents Chemother 43:157–60.
Clarke AJ, Dupont C. 1992. O-acetylated peptidoglycan: its occurrence, pathobiological significance, and biosynthesis. Can J Microbiol 38:85–91.

Claverys JP, Martin B. 2003. Bacterial "competence" genes: signatures of active transformation, or only remnants? Trends Microbiol 1:161–5.
Claverys JP, Prudhomme M, Mortier-Barriere I, Martin B. 2000. Adaptation to the environment: *Streptococcus pneumoniae*, a paradigm for recombination-mediated genetic plasticity? Mol Microbiol 35:251–9.
Cleveland J, Montville TJ, Nes IF, Chikindas ML. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. Intl J Food Microbiol 71:1–20.
Cloeckaert A, Baucheron S, Flaujac G, Schwarz S, Kehrenberg C, Martel JL, Chaslus-Dancla E. 2000a. Plasmid-mediated florfenicol resistance encoded by the *floR* gene in *Escherichia coli* isolated from cattle. Antimicrob Agents Chemother 44:2858–60.
Cloeckaert A, Sidi Boumedine K, Flaujac G, Imberechts H, D'Hooghe I, Chaslus-Dancla E. 2000b. Occurrence of a *Salmonella enterica* serovar typhimurium DT104-like antibiotic resistance gene cluster including the floR gene in *S. enterica* serovar agona. Antimicrob Agents Chemother 44:1359–61.
Cloeckaert A, Baucheron S, Chaslus-Dancla E. 2001. Nonenzymatic chloramphenicol resistance mediated by IncC plasmid R55 is encoded by a floR gene variant. Antimicrob Agents Chemother 45:2381–2.
Cloete TE. 2003. Resistance mechanisms of bacteria to antimicrobial compounds. Intl Biodeter Biodegrad 51:277–82.
Cody SH, Abbott SL, Marfin AA, Schulz B, Wagner P, Robbins K, Mohle-Boetani JC, Vugia DJ. 1999. Two outbreaks of multidrug-resistant *Salmonella* serotype typhimurium DT104 infections linked to raw-milk cheese in Northern California. J Am Med Assoc 281:1805–10.
Cohen ML, Tauxe RV. 1986. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science 234:964–9.
Cohen SP, Hooper DC, Wolfson JS, Souza KS, McMurry LM, Levy SB. 1988. Endogenous active efflux of norfloxacin in susceptible *Escherichia coli*. Antimicrob Agents Chemother 32:1187–91.
Cohen SP, McMurry LM, Hooper DC, Wolfson JS, Levy SB. 1989. Cross-resistance to fluoroquinolones in multiple-antibiotic-resistant (Mar) *Escherichia coli* selected by tetracycline or chloramphenicol: decreased drug accumulation associated with membrane changes in addition to OmpF reduction. Antimicrob Agents Chemother 33:1318–25.
Collier CT, Smiricky-Tjardes MR, Abin DM, Wubben JE, Babert VM, Deplanche B, Bane D, Anderson DB, Gaskins HR. 2003. Molecular ecological analysis of porcine ileal microbiota responses to antimicrobial growth promoters. J Anim Sci 81:3035–45.
Collis CM, Hall RM. 1995. Expression of antibiotic resistance genes in the integrated cassettes of integrons. Antimicrob Agents Chemother 39:155–62.
Collis CM, Grammaticopoulos G, Briton J, Stokes HW, Hall RM. 1993. Site-specific insertion of gene cassettes into integrons. Mol Microbiol 9:41–52.
Cooke EM, Kumar PJ, Shooter RA, Rousseau SA, Foulkes AL. 1970. Hospital food as a possible source of *Escherichia coli* in patients. Lancet 1:436–7.
Cooke EM, Sazegar T, Edmondson AS, Brayson JC, Hall D. 1980. *Klebsiella* species in hospital food and kitchens: a source of organisms in the bowel of patients. J Hyg (Lond) 84:97–101.
Cooper TF, Heinemann JA. 2000. Transfer of conjugative plasmids and bacteriophage lambda occurs in the presence of antibiotics that prevent de novo gene expression. Plasmid 43:171–5.
Cooper RK, Starliper CE, Shotts EB, Jr., Taylor PW. 1993. Comparison of plasmids isolated from Romet-30-resistant *Edwardsiella ictaluri* and tribrissen-resistant *Escherichia coli*. J Aquatic Anim Health 5:9–15.
Cotter PD, Guinane CM, Hill C. 2002. The LisRK signal transduction system determines the sensitivity of *Listeria monocytogenes* to nisin and cephalosporins. Antimicrob Agents Chemother 46:2784–90.
Courvalin P. 2005. Antimicrobial drug resistance: prediction is very difficult, especially about the future. Emerg Inf Dis 11(10). Available from: http://www.cdc.gov/ncidod/EID/vol11no10/05-1014.htm. Accessed: June 24, 2002.
Cox LA, Jr., Popken DA. 2004. Quantifying human health risks from virginiamycin used in chickens. Risk Anal 24:271–88.
Cox LA, Popken DA. 2006. Quantifying potential human health impacts of animal antibiotic use: enrofloxacin and macrolides in chickens. Risk Anal 26:135–46.
Crandall AD, Montville TJ. 1998. Nisin resistance in *Listeria monocytogenes* ATCC 700302 is a complex phenotype. Appl Environ Microbiol 64:231–7.
Cromwell GL. 1999. Safety issues, performance benefits of antibiotics of swine examined. Feedstuffs Jun 7:18–21, 24, 33.
Crump JA, Barrett TJ, Nelson JT, Angulo FJ. 2003. Reevaluating fluoroquinolone breakpoints for *Salmonella enterica* serotype Typhi and for non-Typhi salmonellae. Clin Infect Dis 37:75–81.
Dahlberg C, Chao L. 2003. Amelioration of the cost of conjugative plasmid carriage in *Escherichia coli* K12. Genetics 165:1641–9.
[DANMAP] The Danish integrated antimicrobial resistance monitoring and research programme. 2004. DANMAP. Available at: http://www.dfvf.dk/Default.asp?ID=10044.
Dao-Thi MH, Charlier D, Loris R, Maes D, Messens J, Wyns L, Backmann J. 2002. Intricate interactions within the ccd plasmid addiction system. J Biol Chem 277:3733–42.
Davidson PM. 2001. Chemical preservatives and natural antimicrobial compounds. In: Doyle MP, Doyle LRB, Montville TJ, eds. Food microbiology: fundamentals and frontiers. 2nd ed. Washington, DC: American Society for Microbiology. p 593–627.
Davidson PM, Zivanovic S. 2003. The use of natural antimicrobials. In: Zeuthen P, Bøgh-Sørensen L, eds. Food preservation techniques. Cambridge, UK: Woodhead Publishing Ltd. p 5–30.
Davidson PM, Sofos JN, Branen AL. 2005. Antimicrobials in food. Boca Raton: Taylor and Francis.
Davies J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science 264:375–82.
Davis MA, Hancock DD, Besser TE, Rice DH, Gay JM, Gay C, Gearhart L, DiGiacomo R. 1999. Changes in antimicrobial resistance among *Salmonella enterica* Serovar typhimurium isolates from humans and cattle in the Northwestern United States, 1982–1997. Emerg Infect Dis 5:802–6.
Davison HC, Low JC, Woolhouse ME. 2000. What is antibiotic resistance and how can we measure it? Trends Microbiol 8:554–9.
Deckers D, Masschalck B, Aertsen A, Callewaert L, Van Tiggelen CG, Atanassova M, Michiels CW. 2004. Periplasmic lysozyme inhibitor contributes to lysozyme resistance in *Escherichia coli*. Cell Mol Life Sci 61:1229–37.
de Boever EH, Clewell DB, Fraser CM. 2000. *Enterococcus faecalis* conjugative plasmid pAM373: complete nucleotide sequence and genetic analyses of sex pheromone response. Mol Microbiol 37:1327–41.
de Lencastre H, Brown AE, Chung M, Armstrong D, Tomasz A. 1999. Role of transposon Tn*5482* in the epidemiology of vancomycin-resistant *Enterococcus faecium* in the pediatric oncology unit of a New York City Hospital. Microb Drug Resist 5:113–29.
del Solar G, Espinosa M. 2000. Plasmid copy number control: an ever-growing story.Mol Microbiol 37:492–500.
Derrickson-Tharrington E, Kendall PA, Sofos JN. 2005. Inactivation of *Escherichia coli* O157:H7 during storage or drying of apple slices pretreated with acidic solutions.Intl J Food Microbiol 99:79–89.
Dever LA, Dermody TS. 1991. Mechanisms of bacterial resistance to antibiotics.Arch Intern Med 151:886–95.
DeVincent SJ, Viola C.2006. Deliberations of an advisory committee regarding priorities, sources, and methods for collecting animal antimicrobial use data in the United States.Preventive Vet Med 73:133–51.
Dewey CE, Cox BD, Straw BE, Bush EJ, Hurd HS. 1997. Associations between off-label feed additives and farm size, veterinary consultant use, and animal age.Prev Vet Med 31:133–46.
DiPersio PA, Kendall PA, Calicioglu M, Sofos JN. 2003. Inactivation of *Salmonella* during drying and storage of apple slices treated with acidic or sodium metabisulfite solutions.J Food Protect 66:2245–51.
DiPersio PA, Kendall PA, Sofos JN. 2004. Inactivation of *Listeria monocytogenes* during drying and storage of peach slices treated with acidic or sodium metabisulfite solutions.J Food Microbiol 21:641–8.
Dismukes WE. 2000. Introduction to antifungal drugs.Clin Infect Dis 30:653–7.
Doublet B, Lailler R, Meunier D, Brisabois A, Boyd D, Mulvey MR, Chaslus-Dancla E, Cloeckaert A. 2003. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster in *Salmonella enterica* serovar Albany.Emerg Infect Dis 9:585–91.
Doublet B, Weill FX, Fabre L, Chaslus-Dancla E, Cloeckaert A. 2004. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster containing a novel 3'-N-aminoglycoside acetyltransferase gene cassette, aac(3)-Id, in *Salmonella enterica* serovar Newport.Antimicrob Agents Chemother 48:3806–12.
Doucet-Populaire F, Trieu-Cuot P, Dosbaa I, Andremont A, Courvalin P. 1991. Inducible transfer of conjugative transposon Tn*1545* from *Enterococcus faecalis* to *Listeria monocytogenes* in the digestive tracts of gnotobiotic mice. Antimicrob Agents Chemother 35:185–7.
Dunne EF, Fey PD, Kludt P, Reporter R, Mostashari F, Shillam P, Wicklund J, Miller C, Holland B, Stamey K, Barrett TJ, Rasheed JK, Tenover FC, Ribot EM, Angulo FJ. 2000. Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC beta-lactamase. J Am Med Assoc 284:3151–6.
[EARSS] European Antimicrobial Resistance Surveillance System. 2004. Overview of available data in EARSS database. EARSS. Available at: http://www.rivm.nl/earss/Images/EARSS%20annual%20report%202004%20webversie_tcm61-25345.pdf
Earnshaw AM, Lawrence LM. 1998. Sensitivity to commercial disinfectants, and the occurrence of plasmids within various *Listeria monocytogenes* genotypes isolated from poultry products and the poultry processing environment. J Appl Microbiol 84:642–8.
[EC] European Commission. 2002. Opinion on azole antimycotic resistance. EC. Available from: http://europa.eu.int/comm/food/fs/sc/ssc/out278en.pdf.
Egner C, Berg DE. 1981. Excision of transposon Tn*5* is dependent on the inverted repeats but not on the transposase function of Tn*5*. Proc Natl Acad Sci USA 78(1):459–63.
Engster HM, Marvil D, Stewart-Brown B. 2002. The effect of withdrawing growth promoting antibiotics from broiler chickens: a long-term commercial industry study. J Appl Poultry Res 11:431–6.
Engvall A. 2001. May organically farmed animals pose a risk for *Campylobacter* infections in humans? Acta Vet Scand Suppl 95:85–7.
Enne VI, Bennett PM, Livermore DM, Hall LM. 2004. Enhancement of host fitness by the sul2-coding plasmid p9123 in the absence of selective pressure. J Antimicrob Chemother 53:958–63.
Enter-Net. 2003. Enter-net quarterly *Salmonella* report—2003/3, July–September. Available from: http://www.hpa.org.uk/hpa/inter/enter-net/03q3summ.pdf.
[EPA] Environmental Protection Agency. 2005. What are antimicrobial pesticides? Washington DC: EPA. Available from: http://www.epa.gov/oppad001/adinfo.htm.
Erskine RJ, Walker RD, Bolin CA, Bartlett PC, White DG. 2002. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. J Dairy Sci 85:1111–8.
Erskine RJ, Cullor J, Schaellibaum M, Yancey B, Zecconi A. 2004. Bovine mastitis pathogens and trends in resistance to antibacterial drugs. In: NMC Annual Meeting Proceedings. p 400–14.
Evans S, Davies R. 1996. Case control study of multiple-resistant *Salmonella* typhimurium DT104 infection of cattle in Great Britain. Vet Rec 139:557–8.
[FAAIR] facts about antibiotics in animals and the impact on resistance. 2002. FAAIR Scientific Advisory Panel. Select findings and conclusions. In: Barza M, Gorbach SL, eds. The need to improve antimicrobial use in agriculture: ecological and human health consequences. A report of the facts about antibiotics in animals and the impact on resistance (FAAIR) project. Boston Mass: Alliance for the Prudent Use of Antibiotics. Clin Infect Dis 34 (Suppl 3):S73–5.
[FDA] Food and Drug Administration. 1998. Guidance for industry. Guide to minimize microbial food safety hazards for fresh fruits and vegetables. College Park, MD: F DA. Available from: http://vm.dfsan.fda.gov/~dms/prodguid.html.
[FDA/CVM] Food and Drug Administration/Center for Veterinary Medicine. 1998. Approved animal drug list (Green Book). College Park, MD: FDA/CVM. Available from: http://www.fda.gov/cvm/Green_Book/greenbook.html
[FDA/CVM] Food and Drug Administration/Center for Veterinary Medicine. 1999. A proposed framework for evaluating and assuring the human safety of the microbial effects of antimicrobial new animal drugs intended for use in food producing animals. Discussion paper. College Park, MD: FDA/CVM. Fed. Reg. 64(3):887.
[FDA/CVM] Food and Drug Administration/Center for Veterinary Medicine. 2001. Risk assessment of the human health impact of fluoroquinolone resistant *Campylobacter*

associated with the consumption of chicken. College Park, MD: FDA/CVM. Available from: http://www/fda.gov/cvm/antimicrobial/Riskasses.htm.
[FDA/CVM] Food and Drug Administration/Center for Veterinary Medicine. 2002. Guidance for industry: evaluating the safety of antimicrobial new animal drugs with regard to their microbiological effects on bacteria of human health concern. Document no. 152. College Park, MD: FDA/CVM. Available from: http://www/fda/gov/cvm/guidance/dguide152.pdf.
[FDA, USDA] Food and Drug Administration, United States Department of Agriculture. 2000. United States food safety system: precaution in U.S. food safety decisionmaking: annex II to the United States' National Food Safety System Paper. FDA, USDA. Available from: http://www.foodsafety.gov/~fsg/fssyst4.html.
Feighner SD, Dashkevicz MP. 1987. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholyltaurine hydrolase activity. Appl Environ Microbiol 53:331–6.
Ferguson GC, Heinemann JA, Kennedy MA. 2002. Gene transfer between *Salmonella enterica* serovar Typhimurium inside epithelial cells. J Bacteriol 184(8):2235–42.
Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E, Iwen PC, Bradford PA, Angulo FJ, Hinrichs SH. 2000. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. N Engl J Med 342:1242–9.
Finol ML, Marth EH, Lindsay RC. 1982. Depletion of sorbate from different media during growth of Penicillium species. J Food Protect 45:398–404.
Flahaut S, Frere J, Boutibonnes P, Auffray Y. 1997. Relationship between the thermotolerance and the increase of DnaK and GroEL synthesis in *Enterococcus faecalis* ATCC19433. J Basic Microbiol 37:251–8.
Flammer K. 1992. Avian therapeutics. Proceedings of the North American Veterinary Conference. Jan 9–15. Orlando, FLA. 647 p.
Flammer K. 1994. Antimicrobial therapy. In: Ritchie BW, Harrison LR, eds. Avian medicine, principles and application. Lake Worth, Fla.: Wingers Publishing. p 434–456.
Fluit AC, Schmitz FJ. 1999. Class 1 integrons, gene cassettes, mobility, and epidemiology. Eur J Clin Microbiol Infect Dis 18:761–70.
Foreyt WJ, Besser TE, Lonning SM. 2001. Mortality in captive elk from salmonellosis. J Wild Dis 37:399–402.
Forni C, Cascone A, Fiori M, Migliore L. 2002. Sulphadimethoxine and Azolla filiculoides Lam.: a model for drug remediation. Water Res 36:3398–403.
Foster KR, Vecchia P, Repacholi MH. 2000. Risk management: science and the precautionary principle. Science 288(5468):979–81.
Fox MW. 2002. Animal welfare, social progress, and the veterinary profession. J Am Vet Med Assoc 221(11):1550–1.
[FRAC] Fungicide Resistance Action Committee. 2003. FRAC Fungicide List (1). Available from: http://www.frac.info/frac/index.htm. Accessed: June 24, 2002.
Frank JF, Koffi RA. 1990. Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. Food Protect 53(7):550–4.
Frank JF, Chmielewski R. 2001. Influence of surface finish on the cleanability of stainless steel. J Food Protect 64:1178–82.
Franke AE, Clewell DB. 1981. Evidence for a chromosome-borne resistance transposon (Tn*916*) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. J Bacteriol 145(1):494–502.
Franz CM, Holzapfel WH, Stiles ME. 1999. Enterococci at the crossroads of food safety? Intl J Food Microbiol 47:1–24.
Freeman R, Kearns AM, Lightfoot NF. 1994. Heat resistance of nosocomial enterococci. Lancet 344:64–5.
Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender J, Shiferaw B, Reddy S, Ahuja SD, Helfrick DL, Hardnett F, Carter M, Anderson B, Tauxe RV. 2004. Risk factors for sporadic *Campylobacter* infection in the United States: a case-control study in FoodNet sites. Clin Infect Dis 38(Suppl 3):S285–96.
Frost LS, Manchak J. 1998. F- phenocopies: characterization of expression of the F transfer region in stationary phase. Microbiol 144:2579–87.
Gahan CG, O'Driscoll B, Hill C. 1996. Acid adaptation of *Listeria monocytogenes* can enhance survival in acidic foods and during milk fermentation. Appl Environ Microbiol 62:3128–32.
[GAO] General Accounting Office. 1999. Food safety: the agricultural use of antibiotics and its implications for human health. GAO/RCED-99-74. Washington, D.C.: U.S. GAO.
[GAO] General Accounting Office. 2004. Antibiotic resistance. Federal agencies need to better focus efforts to address risk to humans from antibiotic use in animals. GAO-04-490. Washington, D.C.: U.S. GAO. Available from: http://www.gao.gov/new.items/d04490.pdf.
Gaskins HR, Collier CT, Anderson DB. 2002. Antibiotics as growth promotants: mode of action. Anim Biotechnol 13:29–42.
Gaudreau C, Gilbert H. 1998. Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. Antimicrob Agents Chemother 42:2106–8.
Gavalchin J, Katz SE. 1994. The persistence of faecal-borne antibiotics in soil. J Assoc Off Anal Chem Intl 77:481–5.
Gazouli M, Tzelepi E, Markogiannakis A, Legakis NJ, Tzouvelekis LS. 1998. Two novel plasmid-mediated cefotaxime-hydrolyzing beta-lactamases (CTX-M-5 and CTX-M-6) from *Salmonella typhimurium*. FEMS Microbiol Lett 165:289–93.
Ge B, White DG, McDermott PF, Girard W, Zhao S, Hubert S, Meng J. 2003. Antimicrobial-resistant *Campylobacter* species from retail raw meats. Appl Environ Microbiol 69: 3005–7.
George SM, Lund BM, Brocklehurst TF. 1988. The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. Lett Appl Microbiol 6:153–6.
Geornaras I, Belk KE, Scanga JA, Kendall PA, Smith GC, Sofos JN. 2005. Post-processing antimicrobial treatments to control *Listeria monocytogenes* in commercial vacuum packaged bologna and ham stored at 10 °C. J Food Protect 65:116–23.
Gibbons-Burgener SN, Kaneene JB, Lloyd JW, Erskine RJ. 2000. Influence of the milk and dairy beef quality assurance program on dairy farm drug management practices. J Am Vet Med Assoc 216:1960–4.
Giguere S, Prescott JF. 2000. Equine immunity to bacteria. Vet Clin North Am Equine Pract 16:29–47, v-iv.
Gilbert P, McBain AJ. 2003. Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance. Clin Microbiol Rev 16(2):189–208.
Gillespie MT, Lyon BR, Skurray RA. 1989. Gentamycin and antiseptic resistance in epidemic methicillin-resistant *Staphylococcus aureus*. Lancet 1:503.
Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. N Engl J Med 338:1333–8.
Glynn MK, Reddy V, Hutwagner L, Rabatsky-Ehr T, Shiferaw B, Vugia DJ, Segler S, Bender J, Barrett TJ, Angulo FJ. 2004. Prior antimicrobial agent use increases the risk of sporadic infections with multidrug-resistant *Salmonella enterica* serotype Typhimurium: a FoodNet case-control study, 1996–1997. Clin Infect Dis. 38 (Suppl 3):S227–36.
Golding SS, Matthews KR. 2004. Intrinsic mechanism decreases susceptibility of *Escherichia coli* O157:H7 to multiple antibiotics. J Food Protect 67:34–9.
Goldberg JB, Won J, Ohman DE. 1990. Precise excision and instability of the transposon Tn*5* in *Pseudomonas aeruginosa*. J Gen Microbiol 136:789–96.
Goldstein C, Lee MD, Sanchez S, Hudson CR, Phillips B, Register B, Grady M, Liebert C, Summers AO, White DG, Maurer JJ. 2001. Incidence of class 1 and 2 integrases in clinical and normal flora bacteria from livestock, companion animals, and exotics. Antimicrob Agents Chemother 45:723–6.
Goodacre R, Harvey R, Howell SA, Greenham LW, Noble WC. 1997. An epidemiological study of *Staphylococcus intermedius* strains isolated from dogs, their owners and veterinary surgeons. J Anal Appl Pyrolysis 44(1):49–64.
Goryshin IY, Miller JA, Kil YV, Lanzov VA, Reznikoff WS. 1998. Tn*5*/IS*50* target recognition. Proc Natl Acad Sci USA 95:10716–21.
Greene CR. 2001. U.S. organic farming emerges in the 1990s: adoption of certified systems. U.S. Department of Agriculture, Economic Research Service, Resource Economics Division Washington, DC: USDA Economic Research Service.
Greer GG. 2005. Bacteriophage control of foodborne bacteria. J Food Protect 68(5):1102–11.
Gruenewald R, Blum S, Chan J. 1994. Relationship between human immunodeficiency virus infection and salmonellosis in 20- to 59-year-old residents of New York City. Clin Infect Dis 18:358–63.
Guardabassi L, Schwarz S, Lloyd DH. 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother 54(2):321–32.
Guerra B, Soto S, Helmuth R, Mendoza MC. 2002. Characterization of a self-transferable plasmid from *Salmonella enterica* serotype typhimurium clinical isolates carrying two integron-borne gene cassettes together with virulence and drug resistance genes. Antimicrob Agents Chemother 46:2977–81.
Gupta A, Fontana J, Crowe C, Bolstorff B, Stout A, Van Duyne S, Hoekstra MP, Whichard JM, Barrett TJ, Angulo FJ. 2003. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. J Infect Dis 188:1707–16.
Gupta A, Nelson JM, Barrett TJ, Tauxe RV, Rossiter SP, Friedman CR, Joyce KW, Smith KE, Jones TF, Hawkins MA, Shiferaw B, Beebe JL, Vugia DJ, Rabatsky-Ehr T, Benson JA, Root TP, Angulo FJ. 2004. Antimicrobial resistance among *Campylobacter* strains, United States, 1997–2001. Emerg Infect Dis 10:1102–9.
Gustafson RH, Bowen RE. 1997. Antibiotic use in animal agriculture. J Appl Microbiol 83:531–41.
Gustafson JE, Cox SD, Liew YC, Wyllie SG, Warmington JR. 2001. The bacterial multiple antibiotic resistant (Mar) phenotype leads to increased tolerance to tea tree oil. Pathology 33:211–5.
Haapa-Paananen S, Rita H, Savilahti H. 2002. DNA transposition of bacteriophage Mu J Biol Chem 277:2843–51.
Halling-Sorensen B. 2000. Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. Arch Environ Contam Toxicol 40:451–60.
Halling-Sorensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lutzhoft HC, Jorgensen SE. 1998. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere 36:357–93.
Halling-Sorensen B, Lutzhoft HC, Andersen HR, Ingerslev F. 2000. Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. J Antimicrob Chemother 46 (Suppl 1):53–8.
Halling-Sorensen B, Sengelov G, Tjornelund J. 2002. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Arch Environ Contam Toxicol 42:263–71.
Halling-Sorensen B, Sengelov G, Ingerslev F, Jensen LB. 2003. Reduced antimicrobial potencies of oxytetracycline, tylosin, sulfadiazin, streptomycin, ciprofloxacin, and olaquindox due to environmental processes. Arch Environ Contam Toxicol 44:7–16.
Hamilton-Miller JM, Shah S. 2001. Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables. Intl J Antimicrob Agents 18:81–3.
Hamm RM, Hicks RJ, Bemben DA. 1996. Antibiotics and respiratory infections: are patients more satisfied when expectations are met? J Fam Pract 43:56–62.
Hammami A, Arlet G, Ben Redjeb S, Grimont F, Ben Hassen A, Rekik A, Philippon A. 1991. Nosocomial outbreak of acute gastroenteritis in a neonatal intensive care unit in Tunisia caused by multiply drug resistant *Salmonella* producing SHV-2 beta-lactamase. Eur J Clin Microbiol Infect Dis 10:641–6.
Hanlin MB, Kalchayanand N, Ray P, Rak B. 1993. Bacteriocins of lactic acid bacteria in combination have greater antibacterial activity. J Food Protect 56:252–55.
Hansen PK, Lunestad BT, Samuelsen OB. 1992. Effects of oxytetracycline, oxolinic acid and flumequine on bacteria in an artificial marine fish farm sediment. Canadian J Microbiol 38:1307–12.
Hanson ND, Moland ES, Hossain A, Neville SA, Gosbell IB, Thomson KS. 2002. Unusual *Salmonella enterica* serotype Typhimurium isolate producing CMY-7, SHV-9 and OXA-30 beta-lactamases. J Antimicrob Chemother 49:1011–4.
Harris LJ, Fleming HP, Klaenhammer TR. 1991. Sensitivity and resistance of *Listeria monocytogenes* ATCC 19115, Scott A and UAL 500 to nisin. J Food Protect 54:836–40.
Harris LJ, Fleming HP, Klaenhammer TR. 1992. Novel paired starter culture system for sauerkraut, consisting of a nisin-resistant *Leuconostoc mesenteroides* strain and a nisin-producing *Lactococcus lactis* strain. Appl Environ Microbiol 58:1484–9.
Harvey RG, Marples RR, Noble WC. 1994. Nasal carriage of *Staphylococcus intermedius* in humans in contact with dogs. Microb Ecol Health Dis 7(4):225–7.
Hatten LF, Ingram DR, Pittman ST. 2001. Effect of phytase on production parameters and nutrient availability in broilers and laying hens: a review. J Appl Poult Res 10:274–8.

Hawke JP, Thune RL. 1992. Systemic isolation and antimicrobic susceptibility of *Cytophaga columnaris* from commercially reared channel catfish *Ictalurus punctatus*. J Aquatic Anim Health 4:109–13.
Hawke JP, Thune RL, Cooper RK, Judice E, Kelly-Smith M. 2003. Molecular and phenotypic characterization of strains of *Photobacterium damselae* subsp. *piscicida* from hybrid striped bass cultured in Louisiana, USA. J Aquatic Animal Health 15:189–201.
Hayes D, Jensen HH, Fabiosa J. 2002. Technology choice and the economic effects of a ban on the use of antimicrobial feed additives in swine rations. Food Control 13:97–101.
Hayes JR, English LL, Carr LE, Wagner DD, Joseph SW. 2004. Multiple-antibiotic resistance of *Enterococcus* spp. isolated from commercial poultry production environments. Appl Environ Microbiol 70:6005–11.
Hays VW. 1991. Effects of antibiotics. In: Growth regulation in farm animals. Advances in meat research. Vol. 7. Pearson AM, Dutson TR, eds. New York: Elsevier Applied Science. p 299–320.
He X, Chang W, Pierce DL, Seib LO, Wagner J, Fuqua C. 2003. Quorum sensing in *Rhizobium* sp. strain NGR234 regulates conjugal transfer (*tra*) gene expression and influences growth rate. J Bacteriol 185(3):809–22.
Health Canada. 2004. Canadian integrated program for antimicrobial resistance surveillance (CIPARS 2002). Available from: http://www.phac-aspc.gc.ca/cipars-picra/erratum02_e.html. Accessed: June 24, 2002.
Heddle J, Maxwell A. 2002. Quinolone-binding pocket of DNA gyrase: role of GyrB. Antimicrob Agents Chemother 46:1805–15.
Heir E, Sundheim G, Holck AL. 1995. Resistance to quaternary ammonium compounds in *Staphylococcus* spp. isolated from the food industry and nucleotide sequence of the resistance plasmid pST827. J Appl Bacteriol 79:149–56.
Heir E, Sundheim G, Holck AL. 1999. Identification and characterization of quaternary ammonium compound resistant staphylococci from the food industry. Intl J Food Microbiol 48:211–9.
Heir E, Lindstedt BA, Rotterud OJ, Vardund T, Kapperud G, Nesbakken T. 2004a. Molecular epidemiology and disinfectant susceptibility of *Listeria monocytogenes* from meat processing plants and human infections. Int J Food Microbiol 96:85–96.
Heir E, Lindstedt BA, Leegaard TM, Gjernes E, Kapperud G. 2004b. Prevalence and characterization of integrons in blood culture *Enterobacteriaceae* and gastrointestinal *Escherichia coli* in Norway and reporting of a novel class 1 integron-located lincosamide resistance gene. Ann Clin Microbiol Antimicrob 3:12.
Hektoen H, Berge JA, Hormazabal V *et al*. 1995. Persistence of antibacterial agents in marine sediments. Aquaculture 133:175–84.
Helm JD, Hines RK, Hill JE, Caver JA. 1999. Multiple drug-resistant *Salmonella* typhimurium DT104 and DT104b isolated in bobwhite quail (*Colinus virginianus*). Avian Dis 43:788–91.
Helms M, Vastrup P, Gerner-Smidt P, Molbak K. 2002. Excess mortality associated with antimicrobial drug-resistant *Salmonella* typhimurium. Emerg Infect Dis 8:490–5.
Herikstad H, Hayes PS, Hogan J, Floyd P, Snyder L, Angulo FJ. 1997. Ceftriaxone-resistant *Salmonella* in the United States. Pediatr Infect Dis J 16:904–5.
Heuer OE, Pedersen K, Andersen JS, Madsen M. 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. Lett Appl Microbiol 33:269–74.
[HHS/CDC/NCHS] Health and Human Services/Centers for Disease Control and Prevention/National Center for Health Statistics. 2005. Health, United States, 2005. Chartbook on trends of the health of Americans. U.S. Department of HHS/CDCP/NCHS. Hyattsville, MD. Available at: http://www.cdc.gov/nchs/data/hus/hus05.pdf. Accessed: June 24, 2002.
[HHS/FDA/CVM] Health and Human Services/Food and Drug Administration/Center for Veterinary Medicine. 2002. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): NARMS Retail Meat Annual Report. Rockville, Md: Department of HHS/FDA/CVM. Available from: http://www.fda.gov/cvm/coversheet.htm. Accessed: June 24, 2002.
HHS/FDA/CVM. 2003. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): NARMS Retail Meat Annual Report. Rockville, MD: Department of HHS/FDA/CVM. Available from: http://www.fda.gov/cvm/coversheet2003.htm. Accessed: June 24, 2002.
Hof H. 2001. Critical annotations to the use of azole antifungals for plant protection. Antimicrob Agents Chemother 45:2987–90.
Hofacre CL, White DG, Maurer JJ, Morales C, Lobsinger C, Hudson CR, Thayer SG. 2001. Antibiotic resistant bacteria in rendered animal products. Avian Dis 45:953–61.
Hofreuter D, Haas R. 2002. Characterization of two cryptic *Helicobacter pylori* plasmids: a putative source for horizontal gene transfer and gene shuffling. J Bacteriol 184:2755–66.
Holah JT, Taylor JH, Dawson DJ, Hall KE. 2002. Biocide use in the food industry and the disinfectant resistance of persistent strains of *Listeria monocytogenes* and *Escherichia coli*. J Appl Microbiol 92 (Suppl):111S–20S.
Holmberg SD, Solomon SL, Blake PA. 1987. Health and economic impacts of antimicrobial resistance. Rev Infect Dis 9:1065–78.
Holmes AJ, Gillings MR, Nield BS, Mabbutt BC, Nevalainen KM, Stokes HW. 2003. The gene cassette metagenome is a basic resource for bacterial genome evolution. Environ Microbiol 5:383–94.
Holyoak CD, Bracey D, Piper PW, Kuchler K, Coote PJ. 1999. The *Saccharomyces cerevisiae* weak-acid-inducible ABC transporter Pdr12 transports fluorescein and preservative anions from the cytosol by an energy-dependent mechanism. J Bacteriol 181:4644–52.
Hong HJ, Paget MS, Buttner MJ. 2002. A signal transduction system in *Streptomyces coelicolor* that activates the expression of a putative cell wall glycan operon in response to vancomycin and other cell wall-specific antibiotics. Mol Microbiol 44:1199–211.
Hooper DC. 1995. Quinolone mode of action. Drugs. 49 (Suppl 2):10–5.
Hooper DC, Wolfson JS, Souza KS, Ng EY, McHugh GL, Swartz MN. 1989. Mechanisms of quinolone resistance in *Escherichia coli*: characterization of nfxB and cfxB, two mutant resistance loci decreasing norfloxacin accumulation. Antimicrob Agents Chemother 33:283–90.
Horn N, Swindell S, Dodd H, Gasson M. 1991. Nisin biosynthesis genes are encoded by a novel conjugative transposon. Mol Gen Genet 228:129–35.
Hossack DJN, Bird MC, Fowler AA. 1983. The effects of nisin on the sensitivity of microorganisms to antibiotics and other chemotherapeutic agents. In: Woodbine M, ed. Antimicrobials and Agriculture. London: Butterworth. p 425–33.
Huang ES, Stafford RS. 2002. National patterns in the treatment of urinary tract infections in women by ambulatory care physicians. Arch Intern Med 162:41–7.
Hudson CR, Quist C, Lee MD, Keyes K, Dodson SV, Morales C, Sanchez S, White DG, Maurer JJ. 2000. The genetic relatedness of *Salmonella* isolates from nondomestic birds in southeastern United States. J Clin Microbiol 38:1860–5.
Hughes VM, Datta N. 1983. Conjugative plasmids in bacteria of the 'pre-antibiotic' era. Nature 302:725–6.
Humphrey TJ, Richardson NP, Gawler AHL, Allen MJ. 1991. Heat resistance of *Salmonella enteritidis* PT4: the influence of prior exposure to alkaline exposure. Lett Appl Microbiol 12:258–60.
Hunter JE, Shelley JC, Walton JR, Hart C, Bennett M. 1992. Apramycin resistance plasmids in *Escherichia coli*: possible transfer to *Salmonella* typhimurium in calves. Epidemiol Infect 108:271–8.
Hurd HS, Doores S, Hayes D, Mathew A, Maurer J, Silley P, Singer RS, Jones RN. 2004. Public health consequences of macrolide use in food animals: a deterministic risk assessment. J Food Protect 67:980–92.
Hutchinson JM, Foley RN. 1999. Method of physician remuneration and rates of antibiotic prescription. Can Med Assoc J 160:1013–7.
[IFH] International Scientific Forum on Home Hygiene. 2000. Microbial resistance and biocides. A review by the International Scientific Forum on Home Hygiene. Available: http://www.ifh-homehygiene.org/2003/2public.
[IFH] International Scientific Forum on Home Hygiene. 2003. Biocide usage and antimicrobial resistance in home settings: an update. A review by the International Scientific Forum on Home Hygiene. IFH. Available from: http://www.ifh-homehygiene.org/2003/2public/ANTRES_UPDATE.DOC. Accessed: June 19, 2002.
[IFT] Institute of Food Technologists. 2002a. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. A scientific status summary of the Institute of Food Technologists, Chicago, Ill. Davidson PM, Harrison MA, authors. Food Technol 56: 69–78.
[IFT] Institute of Food Technologists. 2002b. Emerging microbiological food safety issues: implications for control in the 21st century. Expert Report. IFT Chicago, Ill. Available from: http://members.ift.org/IFT/Research/IFTExpertReports/microsfs_report.htm. Accessed: June 19, 2002.
Ikeda JS, Samelis J, Kendall PA, Smith GC, Sofos JN. 2003. Acid adaptation does not promote survival or growth of *Listeria monocytogenes* on fresh beef following acid and nonacid decontamination treatments. J Food Protect 66:985–92.
Imberechts H, De Filette M, Wray C, Jones Y, Godard C, Pohl P. 1998. *Salmonella* typhimurium phage type DT104 in Belgian livestock. Vet Rec 143:424–5.
IMS. 2001. IMS HEALTH awarded five-year services contract by U.S. Food and Drug Administration. Available from: http://www.imshealth.com/ims/portal/front/articleC/0,2777,6599_36651_003644,00.html. Accessed: June 19, 2002.
Ingerslev F, Halling-Sorensen B. 2001. Biodegradability of metronidazole, olaquindox, and tylosin and formation of tylosin degradation products in aerobic soil—manure slurries. Ecotoxicol Environ Saf 48:311–20.
[IOM] Institute of Medicine. 1989. Human health risks with the subtherapeutic use of penicillin or tetracyclines in animal feed. Institute of Medicine. Washington DC: National Academy Press.
Jacobsen B, Berglind L. 1998. Persistence of oxytetracycline in sediments from fish farms. Aquaculture 70:365–70.
Jarvis B, Farr J. 1971. Partial purification, specificity and mechanism of action of the nisin-inactivating enzyme from *Bacillus cereus*. Biochim Biophys Acta 227:232–40.
Joerger RD. 2003. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poult Sci 82:640–7.
Johannessen GS, Froseth RB, Solemdal L, Jarp J, Wasteson Y, M Rorvick L. 2004. Influence of bovine manure as fertilizer on the bacteriological quality of organic Iceberg lettuce. J Appl Microbiol 96:787–94.
John JF, Jr., Fishman NO. 1997. Programmatic role of the infectious diseases physician in controlling antimicrobial costs in the hospital. Clin Infect Dis 24:471–85.
Johnson M. 1991. Bacterial resistance to antibiotics: a growing problem in the channel catfish industry. Proceedings of the Louisiana Aquaculture Conference. Baton Rouge, LA. p 22– 3.
Johnston LM, Jaykus LA. 2004. Antimicrobial resistance of *Enterococcus* species isolated from produce. Appl Environ Microbiol 70(5):3133–7.
Jones FT, Ricke SC. 2003. Observations on the history of the development of antimicrobials and their use in poultry feeds. Poult Sci 82:613–7.
Jones TF, Kellum ME, Porter SS, Bell M, Schaffner W. 2002. An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 8:82–4.
Jørgensen SE. 1984. Modeling the fate and effects of toxic substances in the environment. Development in environmental modeling, Vol. 6. Amsterdam: Elsevier. 342 p.
Jorgensen F, Panaretou B, Stephens PJ, Knochel S. 1996. Effect of pre- and post-heat shock temperature on the persistence of thermotolerance and heat shock-induced proteins in *Listeria monocytogenes*. J Appl Bacteriol 80:216–24.
Jukes TH. 1971. The present status and background of antibiotics in the feeding of domestic animals. Ann NY Acad Sci 182:362–79.
Juneja VK, Davidson PM. 1993. Influence of altered fatty acid composition on resistance of *Listeria monocytogenes* to antimicrobials. J Food Protect 56:302–5.
Kallipolitis BH, Ingmer H, Gahan CG, Hill C, Sogaard-Andersen L. 2003. CesRK, a two-component signal transduction system in *Listeria monocytogenes*, responds to the presence of cell wall-acting antibiotics and affects beta-lactam resistance. Antimicrob Agents Chemother 47:3421–9.
Kapperud G, Rorvik LM, Hasseltvedt V, Hoiby EA, Iversen BG, Staveland K, Johnsen G, Leitao J, Herikstad H, Andersson Y, *et al*. 1995. Outbreak of *Shigella sonnei* infection traced to imported iceberg lettuce. J Clin Microbiol 33:609–14.
Kassenborg HD, Hedberg CW, Hoekstra M, Evans MC, Chin AE, Marcus R, Vugia DJ, Smith K, Ahuja SD, Slutsker L, Griffin PM. 2004. Farm visits and undercooked hamburgers as major risk factors for sporadic *Escherichia coli* O157:H7 infection: data from a case-control study in 5 FoodNet sites. Clin Infect Dis 38 (Suppl 3):S271–8.
Kassenborg HD, Smith KE, Vugia DJ, Rabatsky-Ehr T, Bates MR, Carter MA, Dumas NB, Cassidy MP, Marano N, Tauxe RV, Angulo FJ. 2004. Fluoroquinolone-resistant *Campylobacter*

infections: eating poultry outside of the home and foreign travel are risk factors. Clin Infect Dis 38 (Suppl 3):S279–84.
Kearns AM, Freeman R, Lightfoot NF. 1995. Nosocomial enterococci: resistance to heat and sodium hypochlorite. J Hosp Infect 30:193–9.
Kennedy M, Vugia D, Fiorentino T, Farley M, Pass M, Smith K, Smith P, Cieslak P, Griffin P, the EIP FoodNet Working Group. 2000. FoodNet 1996 to 1998: data on deaths and invasive illnesses demonstrate the severity of *Salmonella* and *Listeria*. 2nd International Conference on Emerging Infectious Diseases. Atlanta, Ga.
Keyes K, Hudson C, Maurer JJ, Thayer S, White DG, Lee MD. 2000. Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. Antimicrob Agents Chemother 44:421–4.
Khoo LH. 2001. Annual fish diagnostic laboratory report for 2000. NWAC News 4:6–7.
Kiessling CR, Cutting JH, Loftis M, Kiessling WM, Datta AR, Sofos JN. 2002. Antimicrobial resistance of food-related *Salmonella* isolates, 1999–2000. J Food Protect 65:603–8.
Kiessling CR, Sofos JN, Watts KA, Loftis MH, Kiessling WM, Buen MB, Laster EW, Datta AR. 2004. Antimicrobial resistance of Salmonellae isolated from various products, 1999–2003. Food and Drug Administration, Laboratory Information Bulletin, No. 4330.
Kim E, Aoki T. 1996. Sequence analysis of the florfenicol resistance gene encoded in the transferable R-plasmid of a fish pathogen, *Pasteurella piscicida*. Microbiol Immunol 40: 665–9.
Klein G, Bulte M. 2003. Antibiotic susceptibility pattern of *Escherichia coli* strains with verocytotoxic *E. coli*-associated virulence factors from food and animal species. Food Microbiol 20:27–33.
Klesius PH, Shoemaker CA, Evans JJ. 2000. Efficacy of single and combined *Streptococcus iniae* isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia (*Oreochromis niloticus*). Aquaculture 188 (3–4):237–46.
Klimuszko D, Szynkiewicz M, Piekarowica A, Binek M, Wojcik U. 1989. Transfer of plasmid Hly in vivo in pigs intestine. Comp Immunol Microbiol Infect Dis 12:29–38.
Koeck JL, Arlet G, Philippon A, Basmaciogullari S, Thien HV, Buisson Y, Cavallo JD. 1997. A plasmid-mediated CMY-2 beta-lactamase from an Algerian clinical isolate of *Salmonella* senftenberg. FEMS Microbiol Lett 152:255–60.
Koga T, Hirota N, Takumi K. 1999. Bactericidal activities of essential oils of basil and sage against a range of bacteria and the effect of these essential oils on *Vibrio parahaemolyticus*. Microbiol Res 154:267–73.
Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36:1202–11.
Kotula KL, Kotula AW, Rose BE, Pierson CJ, Camp M. 1997. Reduction of aqueous chlorine by organic material. J Food Protect 60(3):276–82.
Koutsoumanis K, Sofos JN. 2004a. Microbial contamination of carcasses and cuts. In: Jensen WK, ed. Encyclopedia of meat sciences. Amsterdam: Elsevier Academic. p 727–37.
Koutsoumanis KP, Sofos JN. 2004b. Comparative acid stress response of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium after habituation at different pH conditions. Lett Appl Microbiol 38:321–6.
Koutsoumanis KP, Ashton LV, Geornaras I, Belk KE, Scanga JA, Kendall PA, Smith GC, Sofos JN. 2004. Effect of single and sequential hot water and lactic acid decontamination treatments on the survival and growth of *Listeria monocytogenes* and spoilage microflora during aerobic storage of fresh beef at 4, 10, and 25 °C. J Food Protect 67:2703–11.
Koutsoumanis KP, Geornaras I, Sofos JN. 2006. Microbiology of land animals In: Hui YH, ed. Handbook of food science, technology and engineering. Vol. 1, p 52.1–52.43. Boca Raton, FLA.: CRC Press. Taylor and Francis Group.
Kucers A, Bennett N McK. 1987. The use of antibiotics. A comprehensive review with clinical emphasis. 4th ed. London: William Heineman Medical Books.
Kues U, Stahl U. 1989. Replication of plasmids in gram-negative bacteria. Microbiol Rev 53:491–516.
Kummerer K. 2001a. Drugs in the environment: emission of drugs, diagnostic aids, and disinfectants into wastewater by hospitals in relation to other sources—a review. Chemosphere 45:957–69.
Kümmerer K. 2001b. Pharmaceuticals in the environment. Sources, fate, effects and risks. Heidelberg, Germany: Springer.
Kummerer K. 2003. The significance of antibiotics in the environment. J Antimicrob Chemother 52:5–7.
Kummerer K. 2004. Resistance in the environment. J Antimicrob Chemother 54(2):311–20.
Kurenbach B, Bohn C, Prabhu J, Abudukerim M, Szewzyk U, Grohmann E. 2003. Intergeneric transfer of the *Enterococcus faecalis* plasmid pIP501 to *Escherichia coli* and *Streptomyces lividans* and sequence analysis of its tra region. Plasmid 50:86–93.
Lansky PF, Halling-Sorenson B. 1997. The toxic effect of the antibiotic metronidazole on aquatic organisms. Chemosphere 35:2553–61.
Lasley F. 1983. The US poultry industry. Agric Economics Report No. 502. Washington, D.C.: U.S. Department of Agriculture.
Lasley F, Henson W, Jones H. 1983. The US turkey industry. Agric Economics Report No. 525. Washington, D.C.: U.S. Department of Agriculture.
Lau SK, Woo PC, To AP, Lau AT, Yuen KY. 2004. Lack of evidence that DNA in antibiotic preparations is a source of antibiotic resistance genes in bacteria from animal or human sources. Antimicrob Agents Chemother 48:3141–6.
Lear JC, Maillard JY, Dettmar PW, Goddard PA, Russell AD. 2002. Chloroxylenol- and triclosan-tolerant bacteria from industrial sources. J Ind Microbiol Biotechnol 29:238–42.
LeBlanc DJ, Lee LN, Titmas BM, Smith CJ, Tenover FC. 1988. Nucleotide sequence analysis of tetracycline resistance gene tetO from *Streptococcus mutans* DL5. J Bacteriol 170:3618–26.
Lee C, Hu S, Swiatek PJ, Moseley SL, Allen SD, So M. 1985. Isolation of a novel transposon which carries the *Escherichia coli* enterotoxin STII gene. J Bacteriol 162:615–20.
Lee SY, Butler D, Kleckner N. 1987. Efficient Tn*10* transposition into a DNA insertion hot spot in vivo requires the 5-methyl groups of symmetrically disposed thymines within the hot-spot consensus sequence. Proc Natl Acad Sci USA 84:7876–80.
Lee LA, Puhr ND, Maloney EK, Bean NH, Tauxe RV. 1994. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989–1990. J Infect Dis 170:128–34.
Lee MD, Sanchez S, Zimmer M, Idris U, Berrang ME, McDermott PF. 2002. Class 1 integron-associated tobramycin-gentamicin resistance in *Campylobacter jejuni* isolated from the broiler chicken house environment. Antimicrob Agents Chemother 46:3660–4.
Leistner L, Gould GW. 2002. Multiple hurdle technologies. New York: Klywer Academic. 194 p.
Lemaitre JP, Echchannaoui H, Michaut G, Divies C, Rousset A. 1998. Plasmid-mediated resistance to antimicrobial agents among listeriae. J Food Protect 61:1459–64.
Lenski RE, Bouma JE. 1987. Effects of segregation and selection on instability of plasmid pACYC184 in *Escherichia coli* B. J Bacteriol 169:5314–6.
Levy SB. 2001. Antibacterial household products: cause for concern. Emerg Infect Dis 7(3) (Suppl). Available from: http://www.cdc.gov/ncidod/eid/vol7no3_supp/levy.htm.
Lew JF, Swerdlow DL, Dance ME, Griffin PM, Bopp CA, Gillenwater MJ, Mercatante T, Glass RI. 1991. An outbreak of shigellosis aboard a cruise ship caused by a multiple-antibiotic-resistant strain of *Shigella flexneri*. Am J Epidemiol 134:413–20.
Licht TR, Christensen BB, Krogfelt KA, Molin S. 1999. Plasmid transfer in the animal intestine and other dynamic bacterial populations: the role of community structure and environment. Microbiol 145:2615–22.
Licht TR, Laugesen D, Jensen LB, Jacobsen BL. 2002. Transfer of the pheromone-inducible plasmid pCF10 among *Enterococcus faecalis* microorganisms colonizing the intestine of mini-pigs. Appl Environ Microbiol 68(1):187–93.
Liebert CA, Hall RM, Summers AO. 1999. Transposon Tn*21*, flagship of the floating genome. Microbiol Mol Biol Rev 63:507–22.
Lillehaug A, Lunestad BT, Grave K. 2003. Epidemiology of bacterial diseases in Norwegian aquaculture—a description based on antibiotic prescription data for the ten-year peiord 1991–2000. Dis Aquatic Org 53:115–25.
Lin J, Michel LO, Zhang Q. 2002. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrob Agents Chemother 46:2124–31.
Linder JA, Stafford RS. 2001. Antibiotic treatment of adults with sore throat by community primary care physicians: a national survey, 1989–1999. J Am Med Assoc 286:1181–6.
Linton AH. 1977. Antibiotic resistance: the present situation reviewed. Vet Rec 100:354–60.
Lipsitch M, Singer RS, Levin BR. 2002. Antibiotics in agriculture: when is it time to close the barn door?. Proc Natl Acad Sci USA 99:5752–4.
Lodge JK, Weston-Hafer K, Berg DE. 1988. Transposon Tn*5* target specificity: preference for insertion at G/C pairs. Genetics 120:645–50.
Lodge JK, Berg DE. 1990. Mutations that affect Tn*5* insertion into pBR322: importance of local DNA supercoiling. J Bacteriol 172:5956–60.
Longbottom CJ, Carson CF, Hammer KA, Mee BJ, Riley TV. 2004. Tolerance of *Pseudomonas aeruginosa* to *Melaleuca alternifolia* (tea tree) oil is associated with the outer membrane and energy-dependent cellular processes. J Antimicrob Chemother 54:386–92.
Lopes JA. 1998. Susceptibility of antibiotic-resistant and antibiotic-sensitive foodborne pathogens to acid anionic sanitizers. J Food Protect 61:1390–5.
Lorenz MG, Wackernagel W. 1994. Bacterial gene transfer by natural genetic transformation in the environment. Microbiol Rev 58:563–602.
Love DN, Malik R, Norris JM. 2000. Bacteriological warfare amongst cats: what have we learned about cat bite infections? Vet Microbiol 74:179–93.
Low JC, Angus M, Hopkins G, Munro D, Rankin SC. 1997. Antimicrobial resistance of *Salmonella enterica* typhimurium DT104 isolates and investigation of strains with transferable apramycin resistance. Epidemiol Infect 118:97–103.
Lowenthal JW, Lambrecht B, van den Berg TP, Andrew ME, Strom AD, Bean AG. 2000. Avian cytokines—the natural approach to therapeutics. Dev Comp Immunol 24:355–65.
Lu J, Sanchez S, Lee MD, Hofacre C, Harmon BG, Maurer JJ. 2003. Evaluation of broiler litter with reference to the microbial composition as assessed using 16S rDNA and functional gene markers. Appl Environ Microbiol 69:901–8.
Lu K, Asano R, Davies J. 2004. Antimicrobial resistance gene delivery in animal feeds. Emerg Infect Dis 10:679–83.
Luangtongkum T, Morishita TY, Ison AJ, Huan S, McDermott PF, Zhang O. 2006. Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp in poultry. Appl Environ Microbiol 72:3600–7.
Lucey B, Cryan B, O'Halloran F, Wall PG, Buckley T, Fanning S. 2002. Trends in antimicrobial susceptibility among isolates of Campylobacter species in Ireland and the emergence of resistance to ciprofloxacin. Vet Rec 151:317–20.
Lunden J, Autio T, Markkula A, Hellstrom S, Korkeala H. 2003. Adaptive and cross-adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. Intl J Food Microbiol 82:265–72.
Lyon BR, Skurray R. 1987. Antimicrobial resistance of *Staphylococcus aureus:* genetic basis. Microbiol Rev 51:88–134.
Lyon BR, May JW, Skurray RA. 1984. Tn4001: a gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. Mol Gen Genet 193:554–6.
Mackey BM, Derrick C. 1990. Heat shock protein synthesis and thermotolerance in *Salmonella* typhimurium. J Appl Bacteriol 69:373–83.
MacMillan JR. 2003. Drugs used in the U.S. aquaculture industry. Charles Town, W.V.: National Aquaculture Association. Available from: http://www.nationalaquaculture.org/pdf/Drugs%20and%20Chemicals%20in%20US%20Aquaculture%2011.10.pdf. Accessed: June 19, 2002.
MacMillan JR, Schnick R, Fornshell G. 2003. Volume of antibiotics sold in the US domestic aquaculture industry. Charles Town, W.V.: National Aquaculture Association. Available from: http://www.nationalaquaculture.org/pdf/AETF%20Antibiotic%20use%20white%20paper%206.11.03.pdf. Accessed: June 19, 2002.
Macri A, Staza AV, Dojmi di Delupis G. 1998. Acute toxicity of furazolidone on *Artmia salina*, *Daphnia magna*, and *Culex pipiens molestus* Larvae. Ecotoxicol Environ Safety 16: 90–4.
Mahoudeau I, Delabranche X, Prevost G, Monteil H, Piemont Y. 1997. Frequency of isolation of *Staphylococcus intermedius* from humans. J Clin Microbiol 35(8):2153–4.
Makanera A, Arlet G, Gautier V, Manai M. 2003. Molecular epidemiology and characterization of plasmid-encoded beta-lactamases produced by Tunisian clinical isolates of *Salmonella enterica* serotype Mbandaka resistant to broad-spectrum cephalosporins. J Clin Microbiol 41:2940–5.
Makovec JA, Ruegg PL. 2003. Antimicrobial resistance of bacteria isolated from dairy cow milk samples submitted for bacterial culture: 8,905 samples (1994–2001). J Am Vet Med Assoc 222:1582–9.

Marano N, Vugia DJ, Fiorentino T, Segler S, Carter M, Kassenborg H, Smith K, Zansky S, Hollinger K, Angula F, and the EIP FoodNet Working Group. 2000. Fluoroquinolone-resistant *Campylobacter* causes longer duration of diarrhea than fluoroquinolone-susceptible *Campylobacter* strains in FoodNet sites. 2nd International Conference on Emerging Infectious Diseases. Atlanta, GA, July.
Marengo JR, Kok RA, Velagalet R *et al*. 1997. Aerobic degradation of 14C-sarafloxacin hydrochloride in soil. Environ Toxicol Chem 16:462–71.
Marshall CG, Lessard IA, Park I, Wright GD. 1998. Glycopeptide antibiotic resistance genes in glycopeptide-producing organisms. Antimicrob Agents Chemother 42:2215–20.
Martin C, Timm J, Rauzier J, Gomez-Lus R, Davies J, Gicquel B. 1990. Transposition of an antibiotic resistance element in mycobacteria. Nature 345:739–43.
Martin Barrasa JL, Lupiola Gomez P, Gonzalez Lama Z, Tejedor Junco MT. 2000. Antibacterial susceptibility patterns of Pseudomonas strains isolated from chronic canine otitis externa. J Vet Med B Infect Dis Vet Public Health 47(3):191–6.
Martin LJ, Fyfe M, Dore K, Buxton JA, Pollari F, Henry B, Middleton D, Ahmed R, Jamieson F, Ciebin B, McEwen SA, Wilson JB. 2004. Increased burden of illness associated with antimicrobial-resistant *Salmonella enterica* serotype typhimurium infections. J Infect Dis 189:377–84.
Masschalck B, Michiels CW. 2003. Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. Crit Rev Microbiol 29:191–214.
Mason IS, Kietzmann M. 1999. Cephalosporins—pharmacological basis of clinical use in veterinary dermatology. Vet Dermatol 10:187–92.
Mathews KHJ. 2002. The economic effects of an antimicrobial ban in US beef production. J Agric Appl Econ 34:515–30.
Mazel D, Dychinco B, Webb VA, Davies J. 1998. A distinctive class of integron in the *Vibrio cholerae* genome. Science 280:605–8.
Mazzotta AS, Montville TJ. 1997. Nisin induces changes in membrane fatty acid composition of *Listeria monocytogenes* nisin-resistant strains at 10 degrees C and 30 degrees C. J Appl Microbiol 82:32–8.
Mazzotta AS, Montville TJ. 1999. Characterization of fatty acid composition, spore germination, and thermal resistance in a nisin-resistant mutant of *Clostridium botulinum* 169B and in the wild-type strain. Appl Environ Microbiol 65:659–64.
Mazzotta AS, Modi KD, Montville TJ. 2000. Nisin-resistant (Nisr) *Listeria monocytogenes* and Nisr *Clostridium botulinum* are not resistant to common food preservatives. J Food Safety 68:888–90.
McBain AJ, Bartolo RG, Catrenich CE, Charbonneau D, Ledder RG, Price BB, Gilbert P. 2003. Exposure of sink drain microcosms to triclosan: population dynamics and antimicrobial susceptibility. Appl Environ Microbiol 69:5433–42.
McCaig LF, Besser RE, Hughes JM. 2003. Antimicrobial drug prescription in ambulatory care settings, United States, 1992–2000. Emerg Infect Dis 9(4):432–7.
McDonnell G., Denver Russell A. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12(1):147–79.
McEntire JC. 2003. Relationship between nisin resistance and acid sensitivity of *Listeria monocytogenes*. New Brunswick: Rutgers University. Available from: University Microfilms, Ann Arbor, Mich.
McEntire JC, Carman GM, Montville TJ. 2004. Increased ATPase activity is responsible for acid sensitivity of nisin-resistant *Listeria monocytogenes* ATCC 700302. Appl Environ Microbiol 70:2717–21.
McManus. 2000. Antibiotic use and microbial resistance in plant agriculture. ASM News 66(8):448–9.
McManus PS, Stockwell VO, Sundin GW, Jones AL. 2002. Antibiotic use in plant agriculture. Ann Rev Phytopathol 40:443–65.
McMurry L, Petrucci RE, Jr., Levy, SB. 1980. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. Proc Natl Acad Sci USA 77:3974–7.
Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. 1999. Food-related illness and death in the United States. Emerg Infect Dis 5:607–25.
Medrala D, Dabrowski W, Czekajlo-Kolodziej U, Daczkowska-Kozon E, Koronkiewicz A, Augustynowicz E, Manzano M. 2003. The possible effect of a sanitization program on intraspecies differentiation of *Listeria monocytogenes* strains isolated from a fish processing plant. Intl J Hyg Environ Health 206:583–90.
Mellon M, Benbrook C, Benbrook KL. 2001. Hogging it: estimates of antimicrobial abuse in livestock. Cambridge: UCS Publications.
Meng J, Zhao S, Doyle M, Joseph S. 1998. Antibiotic resistance of *Escherichia coli* O157:H7 and O157:NM isolated from animals, food and humans. J Food Protect 61: 1511–4.
Merck. 2003. The Merck veterinary manual. Whitehouse Station, N.J.: Merck and Company. Available from: http://www.merckvetmanual.com/mvm/index.jsp.
Meunier D, Boyd D, Mulvey MR, Baucheron S, Mammina C, Nastasi A, Chaslus-Dancla E, Cloeckaert A. 2002. *Salmonella enterica* serotype Typhimurium DT 104 antibiotic resistance genomic island I in serotype Paratyphi B. Emerg Infect Dis 8:430–3.
Meunier D, Boyd D, Mulvey MR, Baucheron S, Mammina C, Nastasi A, Chaslus-Dancla E, Cloeckaert A. 2002. *Salmonella enterica* serotype Typhimurium DT 104 antibiotic resistance genomic island I in serotype paratyphi B. Emerg Infect Dis 8:430–3.
Migliore L, Cozzolino S, Fiori M. 2000. Phytotoxicity to and uptake of flumequine used in intensive aquaculture on the aquatic weed, *Lythrum salicaria* L. Chemosphere 40: 741–50.
Migliore L, Cozzolino S, Fiori M. 2003. Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere 52:1233–44.
Miller GY, Algozin KA, McNamara PE, Bush EJ. 2003. Productivity and economic effects of antibiotics used for growth promotion in U.S. pork production. J Agric Appl Econ 35:469–82.
Miller GY, McNamara PE, Singer RS. 2006. Stakeholder position paper: economist's perspectives on antibiotic use in animals. Prevent Vet Med 73(2–3):163–8.
Ming X, Daeschel M. 1993. Nisin resistance of foodborne bacteria and the specific resistance responses of *Listeria monocytogenes* Scott A. J Food Protect 56:944–8.
Mizan S, Lee MD, Harmon BG, Tkalcic S, Maurer JJ. 2002. Acquisition of antibiotic resistance plasmids by enterohemorrhagic *Escherichia coli* O157:H7 within rumen fluid. J Food Protect 65:1038–40.
Moir CJ, Eyles MJ. 1992. Inhibition, injury and inactivation of four psychrotrophic foodborne bacteria by the preservatives methyl p-hydroxybenzoate and potassium sorbate. J Food Protect 55:360–6.
Molin S, Tolker-Nielsen T. 2003. Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilization of the biofilm structure. Curr Opin Biotechnol 14:255–61.
Molbak K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydendahl K, Gerner-Smidt P, Petersen AM, Wegener HC. 1999. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype typhimurium DT104. N Engl J Med. 341:1420–5.
Moldave K, Rhodes L. 2003. Bacteriophage emerging as tool in animal health and food safety. Richmond, U.K.: Pharmaprojects. Animal-Pharm 14–16 Jul 18.
Molstad S, Lundborg CS, Karlsson AK, Cars O. 2002. Antibiotic prescription rates vary markedly between 13 European countries. Scand J Infect Dis. 34:366–71.
Monchois V, Abergel C, Sturgis J, Jeudy S, Claverie JM. 2001. *Escherichia coli* ykfE ORFan gene encodes a potent inhibitor of C-type lysozyme. J Biol Chem 276:18437–41.
Mosenthin R, Bauer E. 2000. The potential use of prebiotics in pig nutrition. Asian–Aus J An Sci 13:315–25.
Mosteller TM, Bishop JR. 1993 Sanitizer efficacy against attached bacteria in a milk biofilm. J Food Protect 56:34–41.
Motlagh AM, Bhunia AK, Szostek F, Hansen TR, Johnson MC, Ray B. 1992. Nucleotide and amino acid sequence of pap-gene (pediocin AcH production) in *Pediococcus acidilactici* H. Lett Appl Microbiol 15:45–8.
Mukherjee A, Speh D, Dyck E, Diez-Gonzalez F. 2004. Preharvest evaluation of coliforms, *Escherichia coli, Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. J Food Protect 67:894–900.
Muller AK, Westergaard K, Christensen S, Sorensen SJ. 2002. The diversity and function of soil microbial communities exposed to different disturbances. Microb Ecol 44:49–58.
Muniesa M, Garcia A, Miro E, Mirelis B, Prats G, Jofre J, Navarro F. 2004. Bacteriophages and diffusion of beta-lactamase genes. Emerg Infect Dis 10:1134–7.
Murray NE. 2000. Type I restriction systems: sophisticated molecular machines (a legacy of Bertani and Weigle). Microbiol Mol Biol Rev 64:412–34.
Mustapha A, Liewen MB. 1989. Destruction of *Listeria monocytogenes* by sodium hypochlorite and quaternary ammonium sanitizers. J Food Protect 52:306–11.
Myhal ML, Laux DC, Cohen PS. 1982. Relative colonizing abilities of human fecal and K 12 strains of *Escherichia coli* in the large intestines of streptomycin-treated mice. Eur J Clin Microbiol 1:186–92.
Naas T, Mikami Y, Imai T, Poirel L, Nordmann P. 2001. Characterization of In53, a class 1 plasmid- and composite transposon-located integron of *Escherichia coli* which carries an unusual array of gene cassettes. J Bacteriol 183:235–49.
Nachamkin I, Ung H, Li M. 2002. Increasing fluoroquinolone resistance in *Campylobacter jejuni*, Pennsylvania, USA,1982–2001. Emerg Infect Dis 8:1501–3.
Naderer M, Brust JR, Knowle D, Blumenthal RM. 2002. Mobility of a restriction–modification system revealed by its genetic contexts in three hosts. J Bacteriol 184:2411–9.
Nandi S, Maurer JJ, Hofacre C, Summers AO. 2004. Gram-positive bacteria are a major reservoir of class 1 antibiotic resistance integrons in poultry litter. Proc Natl Acad Sci USA 101:7118–22.
[NARMS] National Antimicrobial Resistance Monitoring System. 2003a. Antimicrobial resistance research unit. National Antimicrobial Resistance Monitoring System. Available from: http://www.arru.saa.ars.usda.gov. Accessed: June 24, 2002.
[NARMS] National Antimicrobial Resistance Monitoring System. 2003b. National antimicrobial resistance monitoring system annual report. Centers for Disease Control and Prevention, Atlanta, Ga. Available from: http://www.cdc.gov/narms/annual/2003/NARMS2003AnnualReport.pdf. Accessed: June 24, 2002.
[NARMS] National Antimicrobial Resistance Monitoring System. 2004. National antimicrobial resistance monitoring system. http://www.fda.gov/cvm/index/narms/narmspg.html. Accessed: June 24, 2002.
[NARMS] National Antimicrobial Resistance Monitoring System. 2006. National antimicrobial resistance monitoring system. Frequently asked questions (FAQ) about antibiotic resistance – which antibiotics used in food-producing animals are related to antibiotics used in humans? http://www.cdc.gov/narms/faq_pages/11.htm. Accessed: June 24, 2002.
Navarro F, Perez-Trallero E, Marimon JM, Aliaga R, Gomariz M, Mirelis B. 2001. CMY-2-producing *Salmonella enterica*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Escherichia coli* strains isolated in Spain (October 1999-December 2000). J Antimicrob Chemother 48:383–9.
[NBA] National Bison Association. 2005. National Bison Association. www.bisoncentral.com. Accessed: June 24, 2002.
[NCCLS] National Committee for Clinical Laboratory Standards. 2002. Clinical and laboratory standards institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard. 2nd ed. NCCLS document M31-A2. Wayne, Penn.: NCCLS.
Nelson JM, Smith KE, Vugia DJ, Rabatsky-Ehr T, Segler SD, Kassenborg HD, Zansky SM, Joyce K, Marano N, Hoekstra RM, Angulo FJ. 2004. Prolonged diarrhea due to ciprofloxacin-resistant campylobacter infection. J Infect Dis 190:1150–7.
Nesvera J, Hochmannova J, Patek M. 1998. An integron of class 1 is present on the plasmid pCG4 from gram-positive bacterium *Corynebacterium glutamicum*. FEMS Microbiol Lett 169(2):391–5.
Neu HC. 1992. The crisis in antibiotic resistance. Science 257:1064–73.
Nield BS, Holmes AJ, Gillings MR, Recchia GD, Mabbutt BC, Nevalainen KM, Stokes HW. 2001. Recovery of new integron classes from environmental DNA. FEMS Microbiol Lett 195:59–65.
Nikaido H, Rosenberg EY, Foulds J. 1983. Porin channels in *Escherichia coli*: studies with beta-lactams in intact cells. J Bacteriol. 153:232–40.
Nikolich MP, Shoemaker NB, Wang GR, Salyers AA. 1994. Characterization of a new type of *Bacteriodes* conjugative transposon, Tcr Emr 7853. J Bacteriol 176:6606–12.
Niwa H, Chuma T, Okamoto K, Itoh K. 2001. Rapid detection of mutations associated with resistance to erythromycin in *Campylobacter jejuni/coli* by PCR and line probe assay. Intl J Antimicrob Agents 18:359–64.
Norgren M, Scott JR. 1991. The presence of conjugative transposon Tn*916* in the recipient strain does not impede transfer of a second copy of the element. J Bacteriol 173:319–24.

NORM/NORM-VET. 2002. Consumption of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromso/Oslo 2003. ISSN: 1502–2307. http://www.unn.no/getfile.php/UNN%20-%20Internett/Fagfolk/www.antibiotikaresistens.no/Dokumenter/normnormvet2009999998.pdf. Accessed: June 24, 2002.
Normand EH, Gibson NR, Reid SW, Carmichael S, Taylor DJ. 2000. Antimicrobial-resistance trends in bacterial isolates from companion-animal community practice in the UK. Prev Vet Med 46:267–78.
Nostro A, Blanco AR, Cannatelli MA, Enea V, Flamini G, Morelli I, Sudano Roccaro A, Alonzo V. 2004. Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. FEMS Microbiol Lett 230:191–5.
Novick RP. 1987. Plasmid incompatibility. Microbiol Rev 51:381–95.
[NPB] National Pork Board. 2005. Take care: use antibiotics responsibly. Des Moines: NPB.
[NRC] National Research Council. 1999. The use of drugs in food animals: benefits and risks. National Research Council. Washington, D.C.: National Academy Press.
[NWAC] National Warmwater Aquaculture Center. 2003. Industry Overview. Thad Cochran National Warmwater Aquaculture Center, Stoneville, Miss: Mississippi State University.
[NWAC] National Warmwater Aquaculture Center. 2004. Annual reports of the fish diagnostic laboratory. Thad Cochran National Warmwater Aquaculture Center, Stoneville, Miss. Mississippi State University Stoneville.
Nygaard K, Lunestad BT, Hektoen H, Berge JA, Hormazabal V. 1992. Resistance to oxytetracycline, oxolinic acid and furazolidone in bacteria from marine sediments. Aquaculture 104:31–6.
O'Driscoll B, Gahan CG, Hill C. 1996. Adaptive acid tolerance response in *Listeria monocytogenes:* isolation of an acid-tolerant mutant which demonstrates increased virulence. Appl Environ Microbiol 62:1693–8.
Ohlsen K, Ziebuhr W, Koller K *et al.* 1998. Effects of sub inhibitory concentrations of antibiotics on alpha-toxin (hla) gene expression of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* isolates. Antimicrob Agents Chemother 42:2817–23.
Odeh R, Kelkar S, Hujer AM, Bonomo RA, Schreckenberger PC, Quinn JP. 2002. Broad resistance due to plasmid-mediated AmpC beta-lactamases in clinical isolates of *Escherichia coli*. Clin Infect Dis 35:140–5.
Ohno T, Kita M, Yamaoka Y, Imamura S, Yamamoto T, Mitsufuji S, Kodama T, Kashima K, Imanishi J. 2003. Antimicrobial activity of essential oils against *Helicobacter pylori*. Helicobacter 8:207–15.
[OIE] Office International des Epizooties. 2004. Terrestrial animal health code. Appendix 3.9.3. Guidelines for the responsible and prudent use of antimicrobial agents in veterinary medicine. Appendix 3.9.4. Risk assessment for antimicrobial resistance arising from the use of antimicrobials in animals. World Organization for Animal Health. Available from: http://www.oie.int/eng/normes/mcode/en-chapitre-3.9.3.htm; http://www.oie.int/eng/normes/mcdoe/en-chapitre-3.9.4.htm.
[OIE] Office International des Epizooties. 2005. 73rd Annual general session of the international committee of the OIE. World Organization for Animal Health. May 22–27, Paris.
Okeke IN, Lamikanra A, Edelman R. 1999. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg Infect Dis 5:18–27.
Onan LJ, la Para TM. 2003. Tylosin-resistant bacteria cultivated from agricultural soil. FEMS Microbiol Lett 220:15–20.
Osterblad M, Pensala O, Peterzens M, Heleniusc H, Huovinen P. 1999. Antimicrobial susceptibility of *Enterobacteriaceae* isolated from vegetables. J Antimicrob Chemother 43: 503–9.
Ozcengiz G, Alaeddinoglu NG. 1991. Bacilysin production by *Bacillus subtilis*: effects of bacilysin, pH and temperature. Folia Microbiol (Praha) 36:522–6.
Paget MS, Leibovitz E, Buttner MJ. 1999. A putative two-component signal transduction system regulates sigmaE, a sigma factor required for normal cell wall integrity in *Streptomyces coelicolor* A3(2). Mol Microbiol 33:97–107.
Paget MS, Chamberlin L, Atrih A, Foster SJ, Buttner MJ. 1999. Evidence that the extracytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). J Bacteriol 181:204–11.
Panagea S, Chadwick PR. 1996. Heat tolerance of vancomycin resistant *Enterococcus faecium*. J Clin Pathol 49:687–9.
Parish ME, Davidson PM. 1993. Methods for evaluation. In: Davidson PM, Branen AL, eds. Antimicrobials in foods. 2nd ed. New York: Marcel Dekker. p597–615.
Patrick DM, Marra F, Hutchinson J, Monnet DL, Ng H, Bowie WR. 2004. Per capita antibiotic consumption: how does a North American jurisdiction compare with Europe? Clin Infect Dis 39:11–7.
Paulson IT, Littlejohn TG, Radstrom P, Sundstrom L, Skold O, Swedberg G, Skurray RA. 1993. The 3′ conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants. Antimicrob Agents Chemother 37:761–8.
Perreten V, Schwarz F, Cresta L, Boeglin M, Dasen G, Teuber M. 1997. Antibiotic resistance spread in food. Nature 389:801–2.
Petersen A, Guardabassi L, Dalsgaard A, Olsen JE. 2000. Class I integrons containing a dhfrI trimethoprim resistance gene cassette in aquatic *Acinetobacter* spp. FEMS Microbiol Lett 182:73–6.
Petersen AD, Walker RD, Bowman MM, Schott HC, Rosser EJ, Jr. 2002. Frequency of isolation and antimicrobial susceptibility patterns of *Staphylococcus intermedius* and *Pseudomonas aeruginosa* isolates from canine skin and ear samples over a 6-year period (1992–1997). J Am Anim Hosp Assoc 38:407–13.
Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, Nightingale C, Preston R, Waddell J. 2004. Does the ruse of antibiotics in food animals pose a risk to human health? A critical review of published data. J Antimic Chemother 53:28–52.
Piccirillo JF, Mager DE, Frisse ME, Brophy RH, Goggin A. 2001. Impact of first-line vs second-line antibiotics for the treatment of acute uncomplicated sinusitis. J Am Med Assoc 286:1849–56.
Piddock LJ. 1995. Quinolone resistance and *Campylobacter* spp. J Antimicrob Chemother 36:891–8.
Pinto-Alphandary H, Mabilat C, Courvalin P. 1990. Emergence of aminoglycoside resistance genes aadA and aadE in the genus *Campylobacter*. Antimicrob Agents Chemother 34:1294–6.
Piper P, Mahe Y, Thompson S, Pandjaitan R, Holyoak C, Egner R, Muhlbauer M, Coote P, Kuchler K. 1998. The pdr12 ABC transporter is required for the development of weak organic acid resistance in yeast. Embo J 17:4257–65.
Pitout JD, Thomson KS, Hanson ND, Ehrhardt AF, Moland ES, Sanders CC. 1998. Beta-lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli,* and *Proteus mirabilis* isolates recovered in South Africa. Antimicrob Agents Chemother 42:1350–4.
Pitout JD, Reisbig MD, Mulvey M, Chui L, Louie M, Crowe L, Church DL, Elsayed S, Gregson D, Ahmed R, Tilley P, Hanson ND. 2003. Association between handling of pet treats and infection with *Salmonella enterica* serotype Newport expressing the AmpC beta-lactamase, CMY-2. J Clin Microbiol 41:4578–82.
Plakas SM, McPhearson RM, Guarina AM. 1988. Disposition and bioavailability of 3H-tetracycline in the channel catfish (*Ictalurus punctatus*). Xenobiotica 1:88–93.
Plumb JA. 1999. Health maintenance and microbial diseases of cultured fishes. Ames: Iowa State University Press.
Podlesek Z, Comino A, Herzog-Velikonja B, Grabnar M. 2000. The role of the bacitracin ABC transporter in bacitracin resistance and collateral detergent sensitivity. FEMS Microbiol Lett 188:103–6.
Pol IE, van Arendonk WG, Mastwijk HC, Krommer J, Smid EJ, Moezelaar R. 2001. Sensitivities of germinating spores and carvacrol-adapted vegetative cells and spores of *Bacillus cereus* to nisin and pulsed-electric-field treatment. Appl Environ Microbiol 67:1693–9.
Ponton J, Ruchel R, Clemons KV, Coleman, DC, Grillot R, Guarro J, Aldebert D, Ambroise-Thomas P, Cano J, Carrillo-Munoz AJ, Gene J, Pinel C, Stevens DA, Sullivan DJ. 2000. Emerging pathogens. Med Mycol 38 (Suppl 1): 225–36.
Poole K, Krebes K, McNally C, Neshat S. 1993. Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. J Bacteriol 175:7363–72.
Posadas BC. 2003a. U.S. aquaculture production: salmon. Available from: http://www.msstate.edu/dept/crec/aquasalmon.html. Accessed: June 24, 2002.
Posadas BC. 2003b. U.S. aquaculture production: tilapia. Available from: http://www.msstate.edu/dept/crec/aquatilapia.html. Accessed: June 24, 2002.
Potenski CJ, Gandhi M, Matthews KR. 2003. Exposure of *Salmonella* Enteritidis to chlorine or food preservatives decreases [corrected] susceptibility to antibiotics. FEMS Microbiol Lett 220:181–6.
Poyart-Salmeron C, Carlier C, Trieu-Cuot P, Courtieu AL, Courvalin P. 1990. Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*cLancet 335:1422–6.
Prazak MA, Murano EA, Mercado I, Acuff GR. 2002. Antimicrobial resistance of *Listeria monocytogenes* isolated from various cabbage farms and packing sheds in Texas. J Food Protect 65:1796–9.
Prescott J. 2000. Antimicrobial drugs: miracle drugs or pig feed? Adv Pork Product Vol. 11. p 37–45. http://www.banffpork.ca/proc/2000pdf/Chap05-Prescott.pdf.
Prescott J, Baggot JD, Walker RD. 2000. Antimicrobial therapy in veterinary medicine. Ames: Iowa State University Press.
Prescott JF, Hanna WJ, Reid-Smith R, Drost K. 2002. Antimicrobial drug use and resistance in dogs. Can Vet J 43:107–16.
Pribil PA, Haniford DB. 2000. Substrate recognition and induced DNA deformation by transposase at the target-capture stage of Tn*10* transposition. J Mol Biol 303:145–59.
Quintiliani R, Jr., Courvalin P. 1996. Characterization of Tn*1547*, a composite transposon flanked by the IS*16* and IS*256*-like elements, that confers vancomycin resistance in *Enterococcus faecalis* BM4281. Gene 172(1):1–8.
Rajashekara G, Haverly E, Halvorson DA, Ferris KE, Lauer DC, Nagaraja KV. 2000. Multidrug-resistant *Salmonella* Typhimurium DT104 in poultry. J Food Protect 63:155–61.
Randall LP, Cooles SW, Osborn MK, Piddock LJ, Woodward MJ. 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. J Antimicrob Chemother 53:208–16.
Rang HP, Dale MM. 1991. Pharmacology. 2nd ed. New York: Churchill Livingstone.
Rankin SC, Aceto H, Cassidy J, Holt J, Young S, Love B, Tewari D, Munro DS, Benson CE. 2002. Molecular characterization of cephalosporin-resistant *Salmonella* enterica serotype Newport isolates from animals in Pennsylvania. J Clin Microbiol 40:4679–84.
Ratnam S, March SB, Ahmed R, Bezanson GS, Kasatiya S. 1988. Characterization of *Escherichia coli* serotype O157:H7. J Clin Microbiol 26:2006–12.
Rawlings DE, Tietze E. 2001. Comparative biology of IncQ and IncQ-like plasmids. Microbiol Mol Biol Rev 65:481–96.
Reid G, Friendship R. 2002. Alternatives to antibiotic use: probiotics for the gut. Anim Biotechnol 13:97–112.
Reznikoff WS. 2003. Tn*5* as a model for understanding DNA transposition. Mol Microbiol 47:1199–1206.
Ribot EM, Wierzba RK, Angulo FJ, Barrett TJ. 2002. *Salmonella enterica* serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990, and 1995. Emerg Infect Dis 8:387–91.
Rickard AH, Lindsay S, Lockwood GB, Gilbert P. 2004. Induction of the mar operon by miscellaneous groceries. J Appl Microbiol 97:1063–8.
Ridley A, Threlfall EJ. 1998. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella* typhimurium DT 104. Microb Drug Resist 4:113–8.
Riesenfeld CS, Goodman RM, Handelsman J. 2004. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. Environ Microbiol 6:981–9.
Roberts MC. 1990. Characterization of the Tet M determinants in urogenital and respiratory bacteria. Antimicrob Agents Chemother 34:476–8.
Roberts MC. 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS Microbiol Rev 19:1–24.
Roe MT, Pillai SD. 2003. Monitoring and identifying antibiotic resistance mechanisms in bacteria. Poult Sci 82:622–6.
Roe MT, Byrd JA, Smith DP, Pillai SD. 2003. Class 1 and 2 integrons in poultry carcasses from broiler house and poultry processing environments. J Food Protect 66:1426–31.
Romanova N, Favrin S, Griffiths MW. 2002. Sensitivity of *Listeria monocytogenes* to sanitizers used in the meat processing industry. Appl Environ Microbiol 68:6405–9.
Ronning, M. 1999. Methodology of drug utilization studies. Available from: http://www.who.int/medicines/library/qsm/icdra99/icdra99util.shtml. Accessed: June 24, 2002.

Rooney RM, Cramer EH, Mantha S, Nichols G, Bartram JK, Farber JM, Benembarek PK. 2004. A review of outbreaks of foodborne disease associated with passenger ships: evidence for risk management. Public Health Rep 119:427–34.
Rosen GD. 2003. Pronutrient antibiotic replacement standards discussed. Feedstuff 75:11.
Russell AD. 1991. Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. J Appl Bacteriol 71:191–201.
Russell AD. 1997. Plasmids and bacterial resistance to biocides. J Appl Microbiol 83(2):155–165.
Russell AD. 2001. Mechanisms of bacterial insusceptibility to biocides. Am J Infect Control 29:259–61.
Russell AD. 2003a. Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. Lancet Infect Dis 3:794–803.
Russell SM. 2003b. The effect of air sacculitis on bird weights, uniformity, fecal contamination, processing errors, and populations of *Campylobacter* spp. and *Escherichia coli*. Poult Sci 82:1326–31.
Russell AD. 2004. Whither triclosan? J Antimicrob Chemother 53:693–5.
Russell AD, Chopra I. 1996. Understanding antibacterial action and resistance, 2nd ed. London: Ellis Horwood.
Russell AD, Furr JR, Maillard JY. 1997. Microbial susceptibility and resistance to biocides. ASMNews 63:481–7.
Safdar A, Armstrong D. 2003. Antimicrobial activities against 84 *Listeria monocytogenes* isolates from patients with systemic listeriosis at a comprehensive cancer center (1955–1997). J Clin Microbiol 41:483–5.
Salyers AA, Shoemaker NB, Stevens AM *et al.* 1995. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. Microbiol Rev 59:579–90.
Samelis J, Sofos JN. 2003a. Strategies to control stress-adapted pathogens and provide safe foods. In: Yousef AE, Juneja VK, eds. Microbial adaptation to stress and safety of new-generation foods. Boca Raton, FLA.: CRC Press, Inc. p 303–51.
Samelis J, Sofos, JN. 2003b. Organic acids. In: Roller, S, ed. Natural antimicrobials for the minimal processing of foods. Cambridge: Woodhead Publishing Limited. p 98–132.
Samelis J, Sofos JN, Kendall PA, Smith GC. 2001a. Influence of the natural microbial flora on acid tolerance response of *Listeria monocytogenes* in a model system of fresh meat decontamination fluids. Appl Environ Microbiol 67:2410–20.
Samelis J, Sofos JN, Kendall PA, Smith GC. 2001b. Fate of *Escherichia coli* O157:H7, *Salmonella* Typhimurium DT104 and *Listeria monocytogenes* in fresh meat decontamination fluids at 4 and 10 °C. J Food Protect 64:950–7.
Samelis J, Sofos JN, Kain ML, Scanga JA, Belk KE, Smith GC. 2001c. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4°C in vacuum packages. J Food Protect 64: 1722–9.
Samelis J, Sofos JN, Ikeda JS, Kendall PA, Smith GC. 2002a. Exposure to non-acid fresh meat decontamination washing fluids sensitizes *Escherichia coli* O157:H7 to organic acids. Lett Appl Microbiol 34:7–12.
Samelis J, Sofos JN, Kendall PA, Smith GC. 2002b. Effect of acid adaptation on survival of *Escherichia coli* O157:H7 in meat decontamination washing fluids and potential effects of organic acid interventions on the microbial ecology of the meat plant environment. J Food Protect 65:33–40.
Samelis J, Sofos JN, Kain ML, Scanga JA, Belk KE, Smith GC. 2002c. Control of *Listeria monocytogenes* with combined antimicrobials following post-process contamination and extended storage of frankfurters at 4°C in vacuum packages. J Food Protect 65:299–307.
Samelis J, Ikeda JS, Sofos JN. 2003. Evaluation of the pH-dependent, stationary-phase acid tolerance of *Listeria monocytogenes* and *Salmonella* Typhimurium DT104 induced by culturing in media with 1% glucose: a comparative study with *Escherichia coli* O157:H7. J Appl Microbiol 95:563–75.
Samelis J, Stopforth JD, Sofos JN. 2004a. Potential for development of stress, adaptation and resistance in pathogenic bacteria found in decontaminated fresh meat environments. IFIS Publishing, FoodInfo Online Features, Feb 11. Available from: http://www.foodsciencecentral.com/library.html#ifis/12883. Accessed: June 24, 2002.
Samelis J, Kendall PA, Smith GC, Sofos JN. 2004b. Acid tolerance of acid- and non-adapted *Escherichia coli* O157:H7 following habituation (10°C) in fresh beef decontamination runoff fluids of different pH values. J Food Protect 67:638–45.
Samelis J, Bedie G, Sofos JN, Belk KE, Scanga JA, Smith GC. 2005a. Combinations of nisin with organic acids or salts to control *Listeria monocytogenes* on sliced pork bologna stored at 4 °C in vacuum packages. Lebensm Wissensch und Technol 38:21–8.
Samelis J, Sofos JN, Kendall PA, Smith GC. 2005b. Survival or growth of *Escherichia coli* O157:H7 in a model system of fresh meat decontamination runoff waste fluids and its resistance to a subsequent lactic acid stress. Appl Environ Microbiol 71:6228–34.
Sampathkumar B, Khachatourians GG, Korber DR. 2004. Treatment of *Salmonella enterica* serovar Enteritidis with a sublethal concentration of trisodium phosphate or alkaline pH induces thermotolerance. Appl Environ Microbiol 70:4613–20.
Sanchez S, Lee MD, Harmon BG, Maurer JJ, Doyle MP. 2002. Animal issues associated with *Escherichia coli* O157:H7. J Am Vet Med Assoc 221:1122–6.
Sanyal D, Greenwood D. 1993. An electron microscope study of glycopeptide antibiotic-resistant strains of *Staphylococcus epidermidis*. J Med Microbiol 39:204–10.
Sargeant JM, Blackwell TE, Martin SW, Tremblay RR. 1994. Production practices, calf health and mortality on six white veal farms in Ontario. Can J Vet Res 58(3):189–95.
Sato K, Bartlett PC, Kaneene JB, Downes FP. 2004a. Comparison of prevalence and antimicrobial susceptibilities of *Campylobacter* spp. isolates from organic and conventional dairy herds in Wisconsin. Appl Environ Microbiol 70:1442–7.
Sato K, Bennedsgaard TW, Bartlett PC, Erskine RJ, Kaneene JB. 2004b. Comparison of antimicrobial susceptibility of *Staphylococcus aureus* isolated from bulk tank milk in organic and conventional dairy herds in the Midwestern United States and Denmark. J Food Protect 67:1104–10.
Savage DC. 1977. Microbial ecology of the gastrointestinal tract. Ann Rev Microbiol 31:107–33.
Schlegelova J, Babak V, Klimova E, Lukasova, J, Navratilova P, Sustackova A, Sediva I, Rysanek D. 2002. Prevalence of and resistance to anti-microbial drugs in selected microbial species isolated from bulk milk samples. J Vet Med Series B 49:216–25.
Schmidt AS, Bruun MS, Dalsgaard I, Larsen JL. 2001. Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile aeromonads from a fish farming environment. Appl Environ Microbiol 67:5675–82.
Schmieger H, Schicklmaier P. 1999. Transduction of multiple drug resistance of *Salmonella enterica* serovar typhimurium DT104. FEMS Microbiol Lett 170:251–6.
Schnabel EL, Jones AL. 1999. Distribution of tetracycline resistance genes and transposons among phylloplane bacteria in Michigan apple orchards. Appl Environ Microbiol 65:4898–907.
Schnappinger D, Hillen W. 1996. Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch Microbiol 165:359–69.
Schneider D, Duperchy E, Depeyrot J, Coursange E, Lenski R, Blot M. 2002. Genomic comparisons among *Escherichia coli* strains B, K-12, and O157:H7 using IS elements as molecular markers. BMC Microbiol 2(1):18.
Schroeder LL, Bullerman LB. 1985. Potential for development of tolerance by *Penicillium digitatum* and *Penicillium italicum* after repeated exposure to potassium sorbate. Appl Environ Microbiol 50:919–23.
Schroeder CM, Zhao C, DebRoy C, Torcolini J, Zhao S, White DG, Wagner DD, McDermott PF, Walker RD, Meng J. 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. Appl Environ Microbiol 68:576–81.
Schroeder CM, White DG, Ge B, Zhang Y, McDermott PF, Ayers S, Zhao S, Meng J. 2003. Isolation of antimicrobial-resistant *Escherichia coli* from retail meats purchased in Greater Washington, DC, USA. Intl J Food Microbiol 85:197–202.
Sengelov G, Agerso Y, Halling-Sorensen B, Baloda SB, Andersen JS, Jensen LB. 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environ Intl 28:587–95.
Senkel IA, Jr., Jolbitado B, Zhang Y, White DG, Ayers S, Meng J. 2003. Isolation and characterization of *Escherichia coli* recovered from Maryland apple cider and the cider production environment. J Food Protect 66:2237–44.
Severina E, Severin A, Tomasz, A. 1998. Antibacterial efficacy of nisin against multidrug-resistant Gram-positive pathogens. J Antimicrob Chemother 41:341–7.
Shaw KJ, Rather PN, Hare RS, Miller GH. 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev 57:138–63.
Sheridan JJ, McDowell, DA. 1998. Factors affecting the emergence of pathogens on foods. Meat Sci 49:S151–67.
Shi L. 2006. DNA microarray-(genome chip)-monitoring the genome chip. Available: www.gene-chips.com. Accessed: June 12, 2002.
Shiferaw B, Shallow S, Marcus R, Segler S, Soderlund D, Hardnett FP, Van Gilder T. 2004. Trends in population-based active surveillance for shigellosis and demographic variability in FoodNet sites, 1996–1999. Clin Infect Dis 38(Suppl 3):S175–80.
Shlaes DM, Gerding DN, John JF, Jr., Craig WA, Bornstein DL, Duncan RA, Eckman MR, Farrer WE, Greene WH, Lorian V, Levy S, McGowan JE, Jr., Paul SM, Ruskin J, Tenover FC, Watanakunakorn C. 1997. Society for healthcare epidemiology of America and infectious diseases society of America joint committee on the prevention of antimicrobial resistance: guidelines for the prevention of antimicrobial resistance in hospitals. Clin Infect Dis 25:584–99.
Shryock T. 2000. Growth promotion and feed antibiotics. In: Prescott JF, Baggot JD, Walker RD, eds. Antimicrobial therapy in veterinary medicine. Ames: Iowa State Press. p 735–43.
Signon L, Kleckner N. 1995. Negative and positive regulation of Tn*10*/IS*10*-promoted recombination by IHF: two distinguishable processes inhibit transposition off of multicopy plasmid replicons and activate chromosomal events that favor evolution of new transposons. Genet Dev 9:1123–36.
Simjee S, White DG, McDermott PF, Wagner DD, Zervos MJ, Donabedian SM, English LL, Hayes JR, Walker RD. 2002. Characterization of Tn1546 in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. J Clin Microbiol 40:4659–65.
Simon O, Jadamus A, Vahjen W. 2001. Probiotic feed additives—effectiveness and expected modes of action. J Anim Feed Sci 10:51–67.
Singh M, O'Hagan DT. 2003. Recent advances in veterinary vaccine adjuvants. Intl J Parasitol 33:469–78.
Sloan A. 2002. The natural and organic foods marketplace. Food Technol 56(1):27–37.
Smalla K, Heuer H, Gotz A, Niemeyer D, Krogerrecklenfort E, Tietze E. 2000. Exogenous isolation of antibiotic resistance plasmids from piggery manure slurries reveals a high prevalence and diversity of IncQ-like plasmids. Appl Environ Microbiol 66:4854–62.
Smets BF, Rittmann BE, Stahl DA. 1993. The specific growth rate of *Pseudomonas putida* PAW1 influences the conjugal transfer rate of the TOL plasmid. Appl Environ Microbiol 59(10):3430–7.
Smith KE, Besser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH, Johnson BP, Moore KA, Osterholm MT. 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992–1998. N Engl J Med 340:1525–32.
Smith DL, Dushoff J, Morris JG. 2005. Agricultural antibiotics and human health. Does antibiotic use in agriculture have a greater impact than hospital use? PLoS Med 2(8): e232.
Snary EL, Kelly LA, Davison HC, Teale CJ, Wooldridge M. 2004. Antimicrobial resistance: a microbial risk assessment perspective. J Antimicrob Chemother 53:906–17.
Sofos JN. 2002a. Stress-adapted, cross-protected, resistant: a concern? Food Technol 56(11):22.
Sofos JN. 2002b. Approaches to pre-harvest food safety assurance. In: Smulders FJM, Collins JD, eds. Food safety assurance and veterinary public health. Vol 1. Food safety assurance in the pre-harvest phase. Wageningen, The Netherlands: Wageningen Academic Publishers. p 23–48.
Sofos JN, Busta FF. 1993. Sorbic acid and sorbates. In: Davidson PM, Branen AL, eds. Antimicrobials in foods. New York: Marcel Dekker.
Sofos JN, Smith GC. 1998. Nonacid meat decontamination technologies: model studies and commercial applications. Intl J Food Microbiol 44:171–88.
Sofos JN, Beuchat LR, Davidson PM, Johnson EA. 1998. Naturally occurring antimicrobials in food. Ames, Ia.: Council for Agricultural Science and Technology. 103 p.

Soto SM, Lobato MJ, Mendoza MC. 2003. Class 1 integron-borne gene cassettes in multidrug-resistant *Yersinia enterocolitica* strains of different phenotypic and genetic types. Antimicrob Agents Chemother 47:421–6.
Spake A, Marcus MB, McGraw D. 1997. O is for outbreak: when a drug resistant Salmonella struck a Vermont farm, health officials knew it might be just the beginning. US News World Report 23:70-9.
Starcic M, Zgur-Bertok D, Jordi BJ, Wosten MM, Gaastra W, van Putten JP. 2003. The cyclic AMP-cyclic AMP receptor protein complex regulates activity of the *traJ* promoter of the *Escherichia coli* conjugative plasmid pRK100. J Bacteriol 185(5):1616–23.
Starliper CE, Cooper RK, Shotts EB, Jr., Taylor PW. 1998. Plasmid-mediated Romet resistance of *Edwardsiella ictaluri*. J Aquatic Anim Health 5:1–8.
Stein DC, Gregoire S, Piekarowicz A. 1988. Restriction of plasmid DNA during transformation but not conjugation in *Neisseria gonorrhoeae*. Infect Immunol 56:112–6.
Steinman MA, Gonzales R, Linder JA, Landefeld CS. 2003. Changing use of antibiotics in community-based outpatient practice, 1991–1999. Ann Intern Med 138:525–33.
Sternberg NL, Maurer R. 1991. Bacteriophage-mediated generalized transduction in *Escherichia coli* and Salmonella typhimurium. In: Miller JH, ed., Bacterial genetic systems. Meth Enzymol 204. New York: Academic Press. p 18–42.
Sternberg S. 1999. Antimicrobial resistance in bacteria from pets and horses. Acta Vet Scand Suppl 92:37–50.
Stokes HW, Hall RM. 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol 3:1669–83.
Stokes HW, O'Gorman B, Recchia GD, Parsekhian M, Hall RM. 1997. Structure and function of 59-base element recombination sites associated with mobile gene cassettes. Mol Microbiol 26:731–45.
Stokes HW, Holmes AJ, Nield BS, Holley MP, Nevalainen KM, Mabbutt BC, Gillings M. 2001. Gene cassette PCR: sequence-independent recovery of entire genes from environmental DNA. Appl Environ Microbiol 67:5240–6.
Stopforth JD, Sofos JN. 2006. Recent advances in pre- and post-slaughter intervention strategies for control of meat contamination. In: Juneja VJ, Cherry JP, Tunick MH, eds. Advances in microbial food safety. ACS Symposium 931. Recent advances in intervention strategies to improve food safety. American Chemical Society. Washington, D.C.: Oxford University Press.
Stopforth JD, Samelis J, Sofos JN, Kendall PA, Smith GC. 2002. Biofilm formation by acid-adapted and nonadapted *Listeria monocytogenes* in fresh beef decontamination washings and its subsequent inactivation with sanitizers. J Food Protect 65:1717–27.
Stopforth JD, Samelis J, Sofos JN, Kendall PA, Smith GC. 2003a. Influence of organic acid concentration on survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in beef carcass wash water and on model equipment surfaces. Food Microbiol 20:651–60.
Stopforth JD, Samelis J, Sofos JN, Kendall PA, Smith GC. 2003b. Influence of extended acid stressing in fresh beef decontamination runoff fluids on sanitizer resistance of acid-adapted *Escherichia coli* O157:H7 in biofilms. J Food Protect 66:2258–66.
Stopforth JD, Ikeda JS, Kendall PA, Sofos JN. 2004a. Survival of acid-adapted or nonadapted *Escherichia coli* O157:H7 in apple wounds and surrounding tissue following chemical treatments and storage. Intl J Food Microbiol 90:51–61.
Stopforth JD, Yoon Y, Belk KE, Scanga JA, Kendall PA, Smith GC, Sofos JN. 2004b. Effect of simulated spray-chilling with chemical solutions on acid-habituated and non-acid-habituated *Escherichia coli* O157:H7 cells attached to beef carcass tissue. J Food Protect 67:2099–106.
Stopforth JD, Yoon Y, Barmpalia IM, Samelis J, Skandamis PN, Sofos JN. 2005. Reduction of *Listeria monocytogenes* populations during exposure to a simulated gastric fluid following storage of inoculated frankfurters formulated and treated with preservatives. Intl J Food Microbiol 99:309–19.
Streatfield SJ, Howard JA. 2003. Plant-based vaccines. Intl J Parasitol 33:479–93.
Summers AO. 2002. Generally overlooked fundamentals of bacterial genetics and ecology. Clin Infect Dis 34(Suppl 3):S85–92.
Sundheim G, Hagtvedt T, Dainty R. 1992 Resistance of meat associated staphylococci to a quaternary ammonium compound. Food Microbiol 9:161–7.
Sundin GW. 2002. Distinct recent lineages of the strA- strB streptomycin-resistance genes in clinical and environmental bacteria. Curr Microbiol 45:63–9.
Sundrum A. 2001. Organic livestock farming. A critical review. 67:207–15.
[SVARM] Swedish veterinary antimicrobial resistance monitoring. 2002. Swedish veterinary antimicrobial resistance monitoring. Uppsala, Sweden: National Veterinary Institute. Available from: http://www.sva.se/pdf/svarm2002.pdf.
Swartz MN. 2002. Human diseases caused by foodborne pathogens of animal origin. Clin Infect Dis 34(Suppl 3):S111–22.
Szabo M, Kiss J, Kotany G, Olasz F. 1999. Importance of illegitimate recombination and transposition in IS*30*-associated excision events. Plasmid 42:192–209.
Szybalski W. 1953. Genetic studies on microbial cross resistance to toxic agents.II. Cross resistance of Micrococcus pyogenes var. aureus to thirty-four antimicrobial agents. Antibiotics Chemother 3:1095–103.
Taglicht D, Padan E, Oppenheim AB, Schuldiner S. 1987. An alkaline shift induces the heat shock response in *Escherichia coli*. J Bacteriol 169:885–7.
Takashima N, Aoki T, Kitao T. 1985. Epidemiological surveillance of drug resistant strains of *Pasturella piscicida*. Fish Pathol 20:209–17.
Talan DA, Staatz D, Staatz A, Overturf GD. 1989. Frequency of *Staphylococcus intermedius* as human nasopharyngeal flora. J Clin Microbiol 27:2393.
Tamaki S, Matsuhashi M. 1973. Increase in sensitivity to antibiotics and lysozyme on deletion of lipopolysaccharides in *Escherichia coli* strains. J Bacteriol 114:453–4.
Tan HM. 1999. Bacterial catabolic transposons. Appl Microbiol Biotechnol 51:1–12.
Tan MW. 2002. Cross-species infections and their analysis. Ann Rev Microbiol 56:539–65.
Tankovic J, Perichon B, Duval J, Courvalin P. 1996. Contribution of mutations in gyrA and parC genes to fluoroquinolone resistance of mutants of *Streptococcus pneumoniae* obtained in vivo and in vitro. Antimicrob Agents Chemother 40:2505–10.
Tanner MA, Everett CL, Youvan DC. 2000. Molecular phylogenetic evidence for noninvasive zoonotic transmission of *Staphylococcus intermedius* from a canine pet to a human. J Clin Microbiol 38:1628–31.
Taormina PJ, Beuchat LR. 2001. Survival and heat resistance of *Listeria monocytogenes* after exposure to alkali and chlorine. Appl Environ Microbiol 67(6):2555–63.
Taormina PJ, Beuchat LR. 2002. Survival of *Listeria monocytogenes* in commercial food-processing equipment cleaning solutions and subsequent sensitivity to sanitizers and heat. J Appl Microbiol 92:71–80.
Taormina PJ, Beuchat LR, Slutsker L. 1999. Infections associated with eating seed sprouts: an international concern. Emerg Infect Dis 5(5). Available from: http://www.cdc.gov/ncidod/eid/vol5no5/taormina.htm.
Tauxe RV, Cavanaugh TR, Cohen ML. 1989. Interspecies gene transfer in vivo producing an outbreak of multiply resistant shigellosis. J Infect Dis 160:1067–70.
Tauxe RV, Puhr ND, Wells JG, Hargrett-Bean N, Blake PA. 1990. Antimicrobial resistance of *Shigella* isolates in the USA: the importance of international travelers. J Infect Dis 162:1107–11.
Taylor LH, Latham SM, Woolhouse ME. 2001. Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci. 356:983–9.
Teuber M, Meile L, Schwarz F. 1999. Acquired antibiotic resistance in lactic acid bacteria from food. Antonie Van Leeuwenhoek 76:115–37.
Thamsborg SM. 2001. Organic farming in the Nordic countries—animal health and production. Acta Vet Scand Suppl 95:7–15.
Threlfall EJ, Frost JA, Ward LR, Rowe B. 1996. Increasing spectrum of resistance in multiresistant *Salmonella* Typhimurium. Lancet 347:1053–4.
Threlfall EJ, Hampton MD, Chart H, Hopkins KL, Ward LR, Tebbutt G. 2004. Emergence of new subclones of multiresistant *Salmonella* Typhimurium DT104 possibly associated with poultry meat. Vet Rec 154:89–90.
Tikofsky LL, Barlow JW, Santisteban C, Schukken YH. 2003. A comparison of antimicrobial susceptibility patterns for *Staphylococcus aureus* in organic and conventional dairy herds. Microb Drug Resist 9(Suppl 1):S39–45.
Tomich PK, An FY, Clewell DB. 1980. Properties of erythromycin-inducible transposon Tn*917* in *Streptococcus faecalis*. J Bacteriol 141:1366–74.
Tompkin RB. 2002. Control of *Listeria monocytogenes* in the food-processing environment. J Food Protect 65:709–25.
Tribble GD, Parker AC, Smith CJ. 1999. Genetic structure and transcriptional analysis of a mobilizable, antibiotic resistance transposon from *Bacteriodes*. Plasmid 41:1–12.
Ultee A, Kets EP, Alberda M, Hoekstra FA, Smid EJ. 2000. Adaptation of the food-borne pathogen *Bacillus cereus* to carvacrol. Arch Microbiol 174:233–8.
Unicomb LE, Ferguson J, Stafford RJ, Ashbolt R, Kirk MD, Becker NG, Patel MS, Gilbert GL, Valcanis M, Mickan L. 2006. Low-level fluoroquinolone resistance among *Campylobacter jejuni* isolates in Australia. Clin Infect Dis 42:1368–74.
[USDA] U.S. Department of Agriculture. 2000. Feedlot '99 Part II: baseline reference of feedlot health and health management 1999. Washington, D.C. U.S. Department of Agriculture. Available from: http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/feedlot/Feedlot99/FD99pt2.pdf.
[USDA] U.S. Department of Agriculture. 2002. US dairy herd structure. Washington, D.C.: U.S. Department of Agriculture. Available from: http://usda.mannlib.cornell.edu/reports/nassr/livestock/dairy-herd/specda02.txt.
[USDA] U.S. Department of Agriculture. 2003. US hog breeding herd structure. Washington, D.C.: U.S. Department of Agriculture. Available from: http://usda.mannlib.cornell.edu/reports/nassr/livestock/hog-herd/spehog02.pdf.
[USDA] U.S. Department of Agriculture. 2004a. Trout production. Washington, D.C.: U.S. Department of Agriculture. Available from: http://usda.mannlib.cornell.edu/reports/nassr/other/ztp-bb/trpr0204.txt.
[USDA] U.S. Department of Agriculture. 2004b. Agricultural chemical usage: 2003 fruit summary. Washington, D.C.: USDA. Available from: http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agcf0804.txt.
[USDA] U.S. Department of Agriculture. 2005a. Livestock slaughter. 2004 summary. Washington, D.C.: U.S. Department of Agriculture. Available from: http://usda.mannlib.cornell.edu/reports/nassr/livestock/pls-bban/lsan0305.pdf.
[USDA] U.S. Department of Agriculture. 2005b. Sheep and goats. Washington, D.C.: USDA. Available from: http://usda.mannlib.cornell.edu/reports/nassr/livestock/pgg-bb/shep0105.txt.
[USDA/ARS] U.S. Department of Agriculture/Agricultural Research Service. 2006. NARMS reports. Beltsville, Md.: USDA/ARS. Available from: http://www.ars.usda.gov/Main/docs.htm?docid=6750.
[USDA/FSIS] U.S. Department of Agriculture/Food Safety and Inspection Service. 1996. Pathogen reduction; Hazard analysis and critical control point (HACCP) systems; final rule. Federal Register. 25:9 CFR Part 304.
[USDA/FSIS] U.S. Department of Agriculture/Food Safety and Inspection Service. 2000. Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate and potassium lactate. Washington, D.C.: USDA/FSIS. Fed. Reg. Mar. 31:17128.
[USDA/FSIS] U.S. Department of Agriculture/Food Safety and Inspection Service. 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; final rule. Washington, D.C.: USDA/FSIS. Fed. Reg. Jun 6:34208.
[USDA/NASS] U.S. Department of Agriculture/National Agricultural Statistics Service. 2002. Agricultural Statistics Database. United States Department of Agricultural Statistics Database—1920 to 2001—U.S. totals (online) http://www.nass.usda.gov/research/.
[USDA/NASS] U.S. Department of Agriculture/National Agricultural Statistics Service. 2005. Poultry—Production and value. 2004 summary. Washington, D.C.: USDA/NASS. Available from: http://usda.mannlib.cornell.edu/reports/nassr/poultry/pbh-bbp/.
USDHHS, AHRQ, HCFA, HRSA, DA, DOD, DVA, EPA. 2001. A public health action plan to combat antimicrobial resistance. Part 1: Domestic Issues. Interagency Task Force on Antimicrobial Resistance. Centers for Disease Control, Food and Drug Administration, and National Institutes of Health of the United States Department of Health and Human Services, Agency for Healthcare Research and Quality, Health Care Financing Administration, Health Resources and Services Administration, Department of Agriculture, Department of Defense, Department of Veterans Affairs, Environmental Protection Agency. Available from: http://www.cdc.gov/drugresistance/actionplan/aractionplan.pdf.
Valkova N, Lepine F, Bollet C, Dupont M, Villemur R. 2002. prbA, a gene coding for an esterase hydrolyzing parabens in *Enterobacter cloacae* and *Enterobacter gergoviae* strains. J Bacteriol 184: 5011–7.

van den Bogaard AE, Stobberingh EE. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs 58:589–607.
Varma JK, Marcus R, Stenzel SA, Hanna SS, Gettner S, Anderson BJ, Hayes T, Shiferaw B, Crume TL, Joyce KW, Angulo FJ. 2004. Risk factors for infection with multi-drug resistant *Salmonella* serotype Newport—United States, 2002–2003, 4th International Conference on Emerging Infectious Diseases. Atlanta, GA. Feb. 29–Mar. 3.
Varma JK, Molbak K, Barrett TJ, Beebe JL, Jones TF, Rabatsky-Ehr T, Smith KE, Vugia DJ, Chang HG, Angulo FJ. 2005. Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. J Infect Dis 191:554–61.
Verstegen MW, Williams BA. 2002. Alternatives to the use of antibiotics as growth promoters for monogastric animals. Anim Biotechnol 13:113–27.
[VMD] Veterinary Medicines Directorate. 2000. Sales of antimicrobial products used as veterinary medicines, growth promoters and coccidiostats in the UK in 2000. Addlestone, Surrey: Vet Med Directorate. Available at: http://www.vmd.gov.uk/.
Vidaver AK. 2002. Uses of antimicrobials in plant agriculture. Clin Infect Dis 34:5107–10.
Vidaver AK. 2005. Antimicrobial use in plant agriculture. In: White DG, Alekshun MN, McDermott PF, eds. Frontiers in antibiotic resistance: a Tribute to Stuart B. Levy. Washington, D.C.: ASM Press.
Villar RG, Macek MD, Simons S, Hayes PS, Goldoft MJ, Lewis JH, Rowan LL, Hursh D, Patnode M, Mead PS. 1999. Investigation of multidrug-resistant *Salmonella* serotype typhimurium DT104 infections linked to raw-milk cheese in Washington State. J Am Med Assoc 281:1811–6.
Vogt DU, Jackson BA. 2001. Antimicrobial resistance: an emerging public health issue. Congressional Research Service. Penny Hill Press: 43.
Waddell CS, Craig NL. 1989. Tn*7* transposition: recognition of the *attTn7* target sequence. Proc Natl Acad Sci USA 86:3958–62.
Walker RD. 2000. The use of fluoroquinolones for companion animal antimicrobial therapy. Aust Vet J 78:84–90.
Wall PG, Morgan D, Lamden K, Ryan M, Griffin M, Threlfall EJ, Ward LR, Rowe B. 1994. A case control study of infection with an epidemic strain of multiresistant *Salmonella* typhimurium DT104 in England and Wales. Commun Dis Rep CDR Rev. 4:R130–5.
Wall PG, Threllfall EJ, Ward LR, Rowe B. 1996. Multiresistant *Salmonella typhimurium* DT104 in cats: a public health risk. Lancet 348:471.
Walsh D, Sheridan JJ, Duffy G, Blair IS, McDowell DA, Harrington D. 2001a. Thermal resistance of wild-type and antibiotic-resistant *Listeria monocytogenes* in meat and potato substrates. J Appl Microbiol 90:555–60.
Walsh D, Duffy G, Sheridan JJ, Blair IS, McDowell, DA. 2001b. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. J Appl Microbiol 90:517–22.
Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL, Bartolo RG. 2003. Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. J Appl Microbiol 94:240–7.
Wang A, Roth JR. 1988. Activation of silent genes by transposons Tn*5* and Tn*10*. Genetics 120:875–85.
Wang Y, Taylor DE. 1990. Natural transformation in *Campylobacter* species. J Bacteriol 172:949–55.
Wang Y, Huang WM, Taylor DE. 1993. Cloning and nucleotide sequence of the *Campylobacter jejuni* gyrA gene and characterization of quinolone resistance mutations. Antimicrob Agents Chemother 37:457–63.
Wang HH, Manuzon M, Lehman M, Wan Kai Luo H, Wittum TE, Yousef A, Bakaletz LO. 2005. Food commensal microbes as a potentially important avenue in transmitting antibiotic resistance genes. FEMS Microbiol. Lett 71:2970–8.
Warren RM, Sampson SL, Richardson M, Van Der Spuy GD, Lombard CJ, Victor TC, van Helden PD. 2000. Mapping of IS*6110* flanking regions in clinical isolates of *Mycobacterium tuberculosis* demonstrates genome plasticity. Mol Microbiol 37:1405–16.
Warth AD. 1977. Mechanism of resistance of *Saccharomyces bailii* to benzoic, sorbic and other weak acids used as food preservatives. J Appl Bacteriol 43:215–30.
Warth AD. 1985. Resistance of yeast species to benzoic and sorbic acids and to sulfur dioxide. J Food Protect 48:564–9.
Warth AD. 1988. Effect of benzoic acid on growth yield of yeasts differing in their resistance to preservatives. Appl Environ Microbiol 54:2091–5.
Watson ADJ, Rosin E. 2000. Antimicrobial drug use in dogs and cats. In: Prescott JF, Baggot JD, Walker RD, eds. Antimicrobial therapy in veterinary medicine. 3rd ed. Ames: Iowa State University Press. p 537–75.
[WCN] World Charter for Nature. 1982. U.N. GA Resolution 37/7. World Charter for Nature.
Weidenmaier C, Kristian SA, Peschel A. 2003. Bacterial resistance to antimicrobial host defenses—an emerging target for novel antiinfective strategies? Curr Drug Targets 4:643–9.
Werner G, Hildebrandt B, Witte W. 2001. Aminoglycoside-streptothricin resistance gene cluster aadE-sat4-aphA-3 disseminated among multiresistant isolates of *Enterococcus faecium*. Antimicrob Agents Chemother 45:3267–9.
Wharton, M, Spiegel RA, Horan JM, Tauxe RV, Wells JG, Barg N, Herndon J, Meriwether RA, MacCormack JN, Levine RH. 1990. A large outbreak of antibiotic-resistant shigellosis at a mass gathering. J Infect Dis 162:1324–8.
[WHO] World Health Organization. 2000. WHO global principles for the containment of antimicrobial resistance in animals intended for food. Report of a WHO consultation, Geneva, Switzerland: WHO. June 2000. Available from: http://www.who.int/emc/diseases/zoo/whoglobalprinciples/index.htm.
[WHO] World Health Organization. 2002. Fact sheet No. 194: antimicrobial resistance. Geneva, Switzerland: WHO. Available from: http://www.who.int/mediacentre/factsheets/fs194/en/.
[WHO] World Health Organization. 2003. Impacts of antimicrobial growth promoter termination in Denmark. Nov 6–9. Foulum, Denmark: WHO. Department of Communicable Diseases, Prevention and Eradication, Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens. Geneva.
Wilkerson C, Samadpour M, van Kirk N, Roberts MC. 2004. Antibiotic resistance and distribution of tetracycline resistance genes in *Escherichia coli* O157:H7 isolates from humans and bovines. Antimicrob Agents Chemother 48:1066–7.
Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. 2001. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob Agents Chemother 45:2716–22.
Yan W, Taylor DE. 1991. Characterization of erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob Agents Chemother 35:1989–96.
Yan JJ, Hong CY, Ko WC, Chen YJ, Tsai SH, Chuang CL, Wu JJ. 2004a. Dissemination of blaCMY-2 among *Escherichia coli* isolates from food animals, retail ground meats, and humans in southern Taiwan. Antimicrob Agents Chemother 48:1353–6.
Yan JJ, Ko WC, Wu HM, Tsai SH, Chuang CL, Wu JJ. 2004b. Complexity of *Klebsiella pneumoniae* isolates resistant to both cephamycins and extended-spectrum cephalosporins at a teaching hospital in Taiwan. J Clin Microbiol 42:5337–40.
Yoon Y, Stopforth JD, Kendall PA, Sofos JN. 2004. Inactivation of *Salmonella* during drying of Roma tomatoes exposed to predrying treatments including peeling, blanching, and dipping in organic acid solutions. J Food Protect 67:1344–52.
Yoon Y, Calicioglu M, Kendall PA, Smith GC, Sofos JN. 2005. Influence of inoculum level and acidic marination on inactivation of *Escherichia coli* O157:H7 during drying and storage of beef jerky. Food Microbiol 22:423–31.
Yousef AE, Juneja VK. 2003. eds. Microbial stress adaptation and food safety. Boca Raton, Fla.: CRC Press. 369 p.
Yu X, Susa M, Knabbe C, Schmid RD, Bachmann TT. 2004. Development and validation of a diagnostic DNA microarray to detect quinolone-resistant *Escherichia coli* among clinical isolates. J Clin Microbiol 42:4083–91.
Zhang G, Ross CR, Blecha F. 2000. Porcine antimicrobial peptides: new prospects for ancient molecules of host defense. Vet Res 31:277–96.
Zhao S, White DG, Ge B, Ayers S, Friedman S, English L, Wagner D, Gaines S, Meng J. 2001a. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. Appl Environ Microbiol 67:1558–64.
Zhao S, White DG, McDermott PF, Friedman S, English L, Ayers S, Meng J, Maurer JJ, Holland R, Walker RD. 2001b. Identification and expression of cephamycinase bla(CMY) genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. Antimicrob Agents Chemother 45:3647–50.
Zhao S, Datta AR, Ayers S, Friedman S, Walker RD, White DG. 2003a. Antimicrobial-resistant *Salmonella* serovars isolated from imported foods. Intl J Food Microbiol 84:87–92.
Zhao S, Qaiyumi S, Friedman S, Singh R, Foley SL, White DG, McDermott PF, Donkar T, Bolin C, Munro S, Baron EJ, Walker RD. 2003b. Characterization of *Salmonella enterica* serotype newport isolated from humans and food animals. J Clin Microbiol 41:5366–71.
Zipperle GF, Jr. Ezzell JW, Jr., Doyle RJ. 1984. Glucosamine substitution and muramidase susceptibility in *Bacillus anthracis*. Can J Microbiol 30:553–9.
Zuccato E, Calamari D, Natangelo M *et al.* 2000. Presence of therapeutic drugs in the environment. Lancet 335:1789–90.

Appendix 1. Use of Antimicrobials in Companion Animals

Antibiotics are used most often in dogs; and, the substances used are often very similar, or identical to those used in humans. Some canine infections, such as pyoderma (bacterial skin inflammation marked by pus-filled lesions) or otitis externa (infection of the external ear canal) require repeated or prolonged therapy. Recurrent pyoderma caused by *Staphylococcus intermedius* is often treated with cephalexin in continuous low-dose or regular pulse therapy (periodic higher dose therapy [Mason and Kietzmann 1999]). Difficult cases are often treated with fluoroquinolones for extended periods (Carlotti and others 1999). Chronic otitis externa, which often involves MDR-drug resistant *Pseudomonas aeroginosa*, is often treated topically with ticarcillin or fluoroquinolones (Martin Barrasa and others 2000; Petersen and others 2002).

The most common infections in cats correspond to wounds (Love and others 2000). Penicillin G is the drug of choice for most skin infections, as well as for acute viral upper respiratory tract infections (with secondary bacterial component), while amoxicillin is the suggested antibiotic treatment for bacterial lower urinary tract infections. Cats are more prone to infections in the oral cavity than dogs and are often treated with amoxicillin, amoxicillin with clavulinic acid, clindamycin, or metronidazole (Watson and Rosin 2000).

Among horses, foals are the most vulnerable to infection; thus, antibiotic use occurs more often in them than in adult horses (Sternberg 1999). Due to the high risk of diarrhea from antibiotics

administered orally, only a narrow range of antibiotics are used in adult horses. Therefore, nonparenteral administration, often by injection, is necessary for treating bacterial disease in horses. In adult horses, penicillin G is suggested for most upper respiratory infections, as well as for abdominal and subcutaneous abscesses; broad-spectrum antibiotics are usually suggested for treating bone and joint conditions; trimethoprim-sulfonamide are recommended for superficial wounds; and, broad-spectrum antibiotics are recommended for deep and contaminated wounds. In neonatal foals, a broad-spectrum antibiotic is recommended, pending culture results (Giguere and Prescott 2000).

Unlike indications for use in poultry, few antibiotics are approved for use in pet birds. Considering the numerous avian species kept as pets and the very small quantities of drugs administered to pet birds, testing of the large majority of antibiotics for approved labeling for use in pet birds is not warranted. Extra-label use is therefore critical to treating infections in pet birds and other minor species. Due to the often advanced immunosuppression of clinically ill birds, rapid progression of potentially fatal diseases, and suspected diagnosis of a mixed bacterial infection, a combination of antibiotics is the empirical treatment choice of treatment in pet birds (Flammer 1992, 1994).

Quantitative usage

Estimates of antimicrobial use in companion animals in the United States are derived from studies that attempt to quantify use in food animals. While the NAHMS contributes information, albeit limited, about antibiotic use in food animals, there is no Federal or private surveillance or monitoring system of antibiotic use in companion animals.

In contrast to the United States, companion animal antibiotic use data is available from the European Union. In the United Kingdom and several other European countries, use of antimicrobials in companion animals represents approximately 6% of the total amount used in animals (Guardabassi and others 2004; VMD 2000). Whether from EU countries or the United States, however, companion animal use estimates most likely underestimate actual use. These figures usually do not include antimicrobials administered to companion animals by veterinarians in clinical settings, those originally purchased for use in food animals, and in the United States, prescriptions for antibiotics indicated for human use that are dispensed from pharmacies. The antibiotics purchased from pharmacies by companion animal owners in the United States for use in their pets are often via the discretionary extra-label policy of the FDA, which enables veterinarians to use drugs for which use or dosage is not in accordance with label indications. There are specific criteria by which veterinarians must abide for extra-label use.

Resistance

In contrast to the substantial amount of literature on antimicrobial resistance in humans and food animals, there is a paucity of information relating to antimicrobial resistance in companion animals (Guardabassi 2004; Prescott and others 2002). Within several studies that have attempted to determine trends in usage and prevalence of resistance among companion animals, there tend to be marked annual variations in data, probably resulting from small sample sizes, changing patterns of use by veterinarians, and differing methods of susceptibility testing among other factors (Sternberg 1999; van den Bogaard and Stobberingh 1999). In the relatively limited number of investigations of antimicrobial use in companion animals, recent studies demonstrate increasing prevalence of resistance (Guardabassi 2004; Normand and others 2000; Prescott and others 2002; Sternberg 1999; Walker 2000). Resistant nosocomial pathogens, including methicillin-resistant *E. faecium*, *Acinetobacter baumannii*, and MDR-uropathogenic *E. coli,* have been reported by several veterinary teaching hospitals (Boerlin and others 2001; Sanchez and others 2002); these organisms are primarily of concern in referral hospitals where more advanced procedures are performed and the patients are more debilitated.

Transfer of resistance to humans

Companion animals, primarily cats and dogs, are potential sources of antimicrobial resistance dissemination, due to the clinical use of antimicrobials in their veterinary medical care and their direct, close contact with humans. The commensal, *S. intermedius*, has appeared with increased frequency in veterinary clinic staff and owners of dogs treated for atopic dermatitis (Harvey and others 1994). In these instances, transmission likely occurs via dogs-to-humans, as *S. intermedius* is rarely isolated in humans (Mahoudeau and others 1997; Talan and others 1989), and strains found in humans correlated with strains found in their dogs (Goodacre and others 1997; Tanner and others 2000). Thus, there is the potential risk that resistance genes from antimicrobial-resistant *S. intermedius* strains in dogs may be transferred to human pathogenic staphylococci. Cefai and others (1994) reported human carriage of methicillin-resistant *S. aureus* (MRSA) linked with a pet dog. Although there is some risk for transfer of fluoroquinolone resistance from companion animals to humans, the risk is difficult to assess and poorly defined (Sternberg 1999). Companion animals, particularly cats on farms, could serve as a source for or recipient of antibiotic-resistant microorganisms from farm animals. Humans were speculated to be the source of vancomycin-resistant *E. faecium* associated with dogs (Simjee 2002).

Appendix 2. Resistance Determinants in Bacteria

Plasmids

Conjugative transfer of DNA between bacteria, especially via specialized organelles called sex pili, was once considered the sole mechanism of transfer of DNA conferring antibiotic resistance (Bower and Daeschel 1999). Following a paradigm shift, it is now believed that the majority of genetic change occurs through transferable plasmid DNA (R Factors) capable of autonomously replicating (duplicating) themselves within bacteria. These R Factors often carry genes that code resistance to multiple antibiotics.

Generally, plasmids are closed circular DNA molecules. These mobile genetic elements vary in their ability to transfer among bacteria, due to the presence of ancillary, unessential, plasmid genes necessary for their mobilization (*oriT*, for example) and physical transfer (*tra* operon) upon cell-to-cell contact between donor and recipient bacteria (Figure 6). Conjugative plasmids also vary in their spectrum of transmission, from narrow (Kues and Stahl 1989) to broad host range (Adamczyk and Jagura-Burdzy 2003; Kurenbach and others 2003; Rawlings and Tietze 2001).

Conjugation is probably the most efficient means for transferring genetic information, especially among disparate bacterial species. Interspecies gene transfer in vivo occurred in association with an outbreak of shigellosis in 1983 (Tauxe and others 1989). The *Shigella* isolate associated with the outbreak carried a plasmid encoding resistance to ampicillin, carbenicillin,

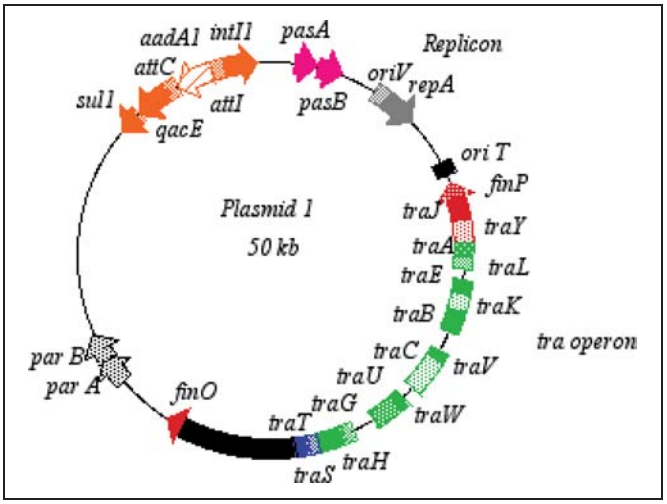

Figure 6—Plasmid. Representative, conjugative plasmid drawn schematically to illustrate basic plasmid replicon (*oriV, repA*) and plasmid segregation genes (*parA,B*), mobilization (*oriT*), and conjugation features (*tra* operon). Included are ancillary genes including those involved in plasmid addiction (*pasA,B*), and antibiotic resistance resident in class 1 integron.

streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole that was identical antimicrobial resistance to an *E. coli* isolate associated with a urinary tract infection of 1 of the case patients occurring prior to the onset of shigellosis.

Hunter and others (1992) addressed the potential for transfer of an apramycin resistance plasmid from *E. coli* to *Salmonella* Typhimurium in calves involved in a salmonellosis outbreak. Prior to antibiotic treatment apramycin-resistant *E. coli* were present, but all *Salmonella* Typhimurium isolates were susceptible. Following treatment, however, apramycin-resistant *Salmonella* Typhimurium were isolated from the calves. Subsequent in vitro experiments demonstrated that plasmids conferring resistance to apramycin could be transferred by conjugation to *Salmonella* Typhimurium. Mizan and others (2002) observed that the acquisition of conjugative R plasmids by *E. coli* O157:H7 from a commensal *E. coli* strain while suspended in rumen fluid, and suggested that the rumen may be a favorable environment for exchange of plasmids between commensals and *E. coli* O157:H7 within the host.

The process of plasmid transfer involves a switch in plasmid replication to rolling circle replication, and transmission of the plasmid as single-stranded DNA to the recipient bacterium (Adamczyk and Jagura-Burdzy 2003). As the recipient cell transforms the plasmid DNA from single to double-stranded form, the DNA becomes hemi-methylated by the bacterial host's methylases, Dam and Dcm, making the recipient plasmid resistant to its host restriction endonucleases, therefore overcoming one important barrier to genetic exchange—restriction (Stein and others 1988). Depending on the microorganism, this modification is not necessarily sufficient in completely resisting the host's type I restriction/modification (R–M) system (Butler and Gotschlich 1991). However, plasmids can counter by either inhibiting the host's restriction defenses or, through selection, having evolved a plasmid genome devoid of the restriction enzyme's target site (Murray and others 2000).

Plasmids are generally classified according to transference (that is, nonconjugative or conjugative) and ability to coexist with other plasmids (known as incompatibility). Conjugation is regulated (Camacho and Casadesus 2002; de Boever and others 2000; Starcic and others 2003), with plasmid transference affected by: (1) growth medium (Ahmer and others 1999), as it impacts on cellular cAMP levels (Starcic and others 2003), and growth rate (Smets and others 1993); (2) cell density (Andrup and others 1999; He and others 2003); (3) growth phase (Frost and Manchak 1998); (4) oxygen tension (Burman 1975); and (5) temperature (Chaslus-Dancla and Lafont 1985; Sherburne 2000). Plasmid transference occurs in situ, within epithelial cells (Ferguson and others 2002), biofilms (Licht and others 1999; Molin and Tolker-Nielsen 2003), or gastrointestinal (Doucet-Populaire and others 1991; Klimuszko and others 1989; Licht and others 2002). Transfer can occur even in the presence of bacteriostatic antibiotics (Cooper and Heinemann 2000). Plasmids encode surface exclusion factors and restriction/modification system(s) that affect the host cell's ability to acquire new plasmids (Anthony and others 1999; De Boever and others 2000; Naderer and others 2002). The mechanism(s) involved in incompatibility result from competition between plasmids regarding replication or partitioning to daughter cells following bacterial cell division (Novick 1987). Therefore, depending on selection pressure, fitness cost, and the benefit (Enne and others 2004) the plasmid provides the cell, plasmids belonging to the same incompatibility group cannot coexist through successive cell divisions following the first introduction of the new plasmid into the recipient bacterial cell. In addition to genes essential to replication (*ori* and *rep*, for example) and partitioning (*par*, for example) to daughter cells, many plasmids contain genes or sequences important to regulation of replication (Chattoraj 2000) and copy number (Chattoraj 2000; del Solar and Espinosa 2000). Unlike genetically engineered, high-copy number (100 copies per cell, for example) plasmids used in molecular biology, most plasmids in nature are present as single or low copy (5–8 copies per cell, for example) (Adamczyk and Jagura-Burdzy 2003). Despite their large size (>100 kb), many of these mobile genetic elements are maintained due to their efficient regulation of plasmid replication (Chattoraj 2000), copy number (Adamczyk and Jagura-Burdzy 2003; Chattoraj 2000; del Solar and Espinosa 2000), and partitioning between daughter cells following cell division (Adamczyk and Jagura-Burdzy 2003).

Plasmids can be maintained in the absence of selection pressure (that is, antibiotic usage) via a "plasmid-addiction" system, wherein the plasmid contains genes that specify "toxin" along with "antidote." Cells that maintain the plasmid are protected while those that lose the plasmid are killed by the plasmid toxin (Dao-Thi and others 2002). Not all plasmids have the ancillary genes necessary for their persistence and ultimate survival within a bacterial population; plasmid loss can occur at a frequency of 0.304/generation. Therefore, without selection pressure to maintain the plasmid within the bacterial population, a plasmid can be completely lost after 30 generations (Lenski and Bouma 1987). Plasmids can coevolve with their bacterial host, however, and be maintained within the bacterial population in the absence of antibiotic selection (Dahlberg and Chao 2003).

Plasmids can provide the bacterial host selective advantages that can ensure the maintenance and survival of the organism (Chu and others 2001; Guerra and others 2002) as well as the plasmid's own survival (Enne and others 2004). Once the resistance gene pool is spread into the indigenous bacteria, there may be a better chance of persistence and mobility, thereby increasing the gene frequency in local populations.

Transposons

Transposons are genetic elements that physically transpose from one genetic position, within the chromosome or plasmid in which they reside, to another. Insertion within a transferable or

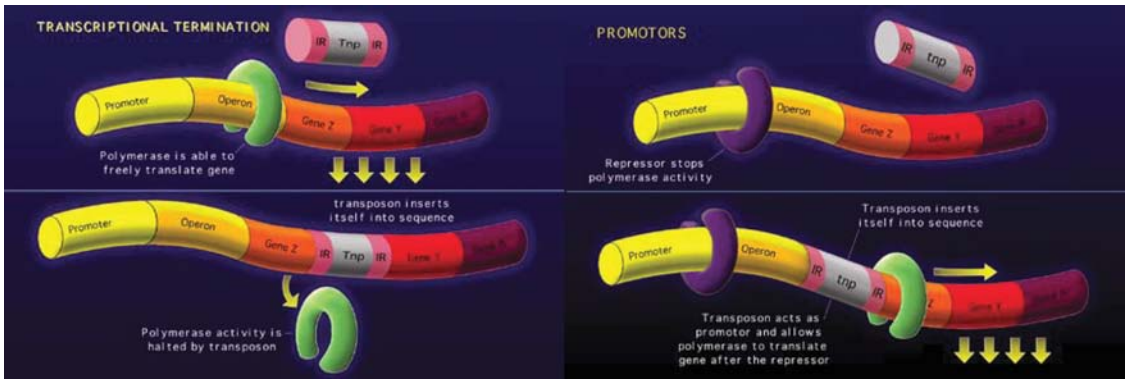

Figure 7—Transposon. Insertion of transposable element can inactivate a gene through its physical insertion into the gene's open reading frame (ORF), the actual sequence that is translated into protein/gene product, or alter gene expression through its insertion into region upstream of its ORF. Transposon's own promoter can then influence transcription of gene(s) downstream of its insertion point.

conjugative plasmid can provide a fortuitous means for dissemination and propagation of a genetic element. "Minimal" transposons, known as insertion sequence (IS) elements, contain just transposase and the inverted, repeat (IR) sequences that flank the element. The transposase recognizes core IR sequence and target sequence into which the transposon inserts itself through a "cut and paste," RecA-independent mechanism (Reznikoff 2003). The transposon can either vacate its current position in the chromosome or plasmid or copy itself during transposition (Berg and others 1984). The excision and insertion rate for transposons vary (Egner and Berg 1981) depending on site of insertion (Egner and Berg 1981), GC (guanine: cytosine) composition (Lodge and others 1988), and chromatin structure (Lee and others 1987; Lodge and Berg 1990; Signon and Kleckner 1995).

The transposon's insertion into a new gene can have the same effect as introduction of a single nucleotide into the gene's open reading frame, causing a frame-shift, inactivating this gene as well as others downstream within the operon (Figure 7). Transposons can also decrease or increase promoter activity, directly or indirectly, by disrupting the promoter sequence, inactivating ancillary genes that regulate promoter activity, or providing a secondary promoter for transcription of gene(s) downstream of the promoter (Wang and Roth 1988). Transposons can also acquire and "mobilize" ancillary genes, creating composite transposons following IS upstream and downstream of bacterial gene(s), obtaining antibiotic resistance (Liebert and others 1999), heavy metal resistance (Liebert and others 1999), and bacteriocin (Horn and others 1991), catabolic (Tan 1999) and virulence genes (Bacciu and others 2004; Lee and others 1985). Transposons associated with antibiotic resistance are composites of IS elements flanking an antibiotic resistance gene (for example, tetracycline resistance transposon, Tn*10*). Unlike plasmids, the same transposon or transposon class can coexist in the same cell in multiple copies, provided multiple copies of the gene(s) borne by the element do(does) not have a detrimental effect on its bacterial host (Norgren and Scott 1991). In addition to the ability of transposons to move genetic information themselves, they can also serve as focal points for recombination that allow re-assortments (Berg and others 1998), rearrangements (Szabo and others 1999), deletions (Szabo and others 1999), and insertions of new and old genetic information, accounting for the genetic plasticity evident in many bacterial species (Hofreuter and Haas 2002; Schneider and others 2002; Warren and others 2000).

Transposons vary with regard to where within a bacterial genome they can insert themselves, which is based on the size of the target recognition site. For "mutator" transposons such as Tn*5* (Goryshin and others 1998), and Tn*10* (Pribil and Haniford 2000), the target sequence is short. For a 5-bp recognition target sequence, a 4000000 bp genome (50% GC content) is expected to contain 3906 random target sites for transposon insertion (Haapa-Paananen and others 2002). Although mutator transposons are expected to insert randomly within a bacterial genome, there are "hot spots" and "cold spots" from transposon insertions (Lee and others 1987). Depending on the bacterial host, these transposons vary in transposition frequency, and rate of excision and/or insertion (Goldberg and others 1990). Other composite transposons such as Tn*7* a have a longer recognition site, effectively having a single insertion site; insertion is, therefore, contingent on the presence of this sequence within the bacterial genome (Waddell and Craig 1989).

There are several composite transposons, where regulation of antibiotic resistance gene(s) is tied into control of transposition (Tomich and others 1980; Tribble and others 1999) and transmission (Tribble and others 1999). Low, inhibitory concentrations of an antibiotic induce expression of both the antibiotic resistance gene and the transposase (Tomich and others 1980), resulting in the subsequent amplification and propagation of the transposable element.

As for plasmids, one class of transposons is capable of conjugation, independent of helper plasmids (Salyers and others 1995). These conjugative transposons are remarkable in their movement across broad phyla, capable of moving between Gram-positive and Gram-negative bacteria (Roberts 1990). Conjugative transposons have been linked to widespread dissemination of resistance to vancomycin (de Lencastre and others 1999; Quintiliani and Courvalin 1996), macrolide, lincosamide, streptogramin B (MLS_B [Chung and others 1999a, b; Roberts 1996b]), and tetracycline (Franke and Clewell 1981; Nikolich and others 1994).

Integrons

Integrons are important catalysts in the development, dissemination, and diversity of multiple drug resistance. They are genetic elements similar to transposons in the possession of a cut–paste recombinase, referred to as the integrase or IntI. Adjacent to *intI*, is an integration site *attI* (Stokes and others 1997). IntI pastes gene(s) into *attI* site that possess the enzyme's target core

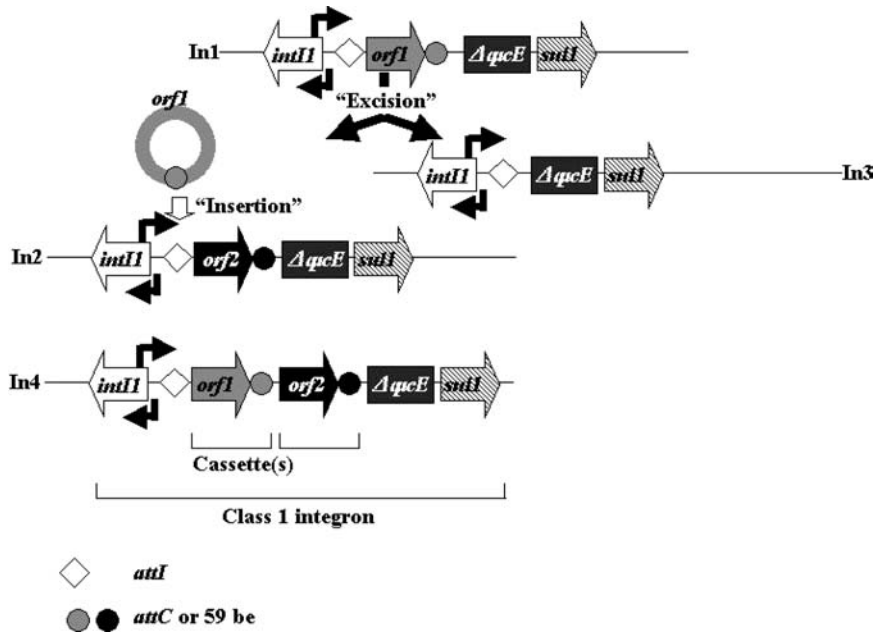

Figure 8—Class 1 Integron. Integrase, IntI1 recognizes *attC* (59 be) sequence of gene cassette and mediates excision of the gene cassette into a circular intermediate (Collis and others 1993) and/or its insertion into *attC* site, producing integrons In3 and In4 shown in this figure.

recognition sequence, GTTRRRY, part of the signature 59 base element (be) sequence of integron gene cassette(s) (Stokes and others 1997). A single integron can acquire multiple genes in tandem, and may contain as many as eight genes (Naas and others 2001). Within *intI1*, there is an internal promoter that drives expression of gene(s) downstream of the integration site *attI* (Collis and others 1995) (Figure 8). Transcription of integron gene cassettes decreases the further the integron gene cassette is from this promoter (Collis and others 1995). Exceptions are those gene cassettes that possess their own promoter (Naas and others 2001).

There are 8 classes of integrons, based on genetic similarities and differences in IntI recombinase. Two of the integron classes—4 and 5—have only been described in vibrios (Mazel and others 1998). Currently, no antibiotic resistance gene cassette(s) have been identified within these integrons (Mazel and others 1998). Integron classes 6 through 8 have been isolated from a soil ecosystem (Nield and others 2001), and several unique gene cassettes have been identified within these "new" integrons, including genes with similarity to aminoglycoside phosphotransferase and RNA methylase. Their contribution to antibiotic resistance, however, is currently unknown (Stokes and others 2001). The three remaining integron classes—1, 2, and 3—are associated with antibiotic resistance (Arakawa and others 1995; Stokes and Hall 1989). The class 1 integrons have been well characterized, and possess additional genes, downstream of the *attI* site and their resident gene cassette(s) which include a functional sulfonamide resistance gene, *sul1* (Stokes and Hall 1989) and partially deleted, nonfunctional quaternary ammonium resistance gene, Δ*qacE* (Paulson and others 1993). The class 1 integrons are, in and of themselves, not mobile, but they do reside on transposons and plasmids (Liebert and others 1999) that ferry them around within the microbial world. It is, therefore, not surprising to find their widespread dissemination in nature (Holmes and others 2003; Nield and others 2001). Integron gene cassettes encode resistances to 6 classes of antibiotics and a disinfectant, quaternary ammonium, representing 51 distinct resistance genes and 9 mechanisms for resistance (Fluit and others 1999). The only resistances not ascribed to integrons are the tetracyclines and several of the Gram-positive-specific antibiotics (for example, vancomycin and streptogramins).

Once believed to be limited in distribution to Gram-negatives, class 1 integrons have now been identified in several Gram-positive bacteria (Clark and others 1999; Martin and others 1990; Nandi and others 2004; Nesvera and others 1998). Class 1 integrons and their associated resistance genes have been identified in clinical (Heir and others 2004b; Soto and others 2003; Zhao and others 2003b) and environmental isolates (Nandi and others 2004; Petersen and others 2000; Roe and others 2003); foodborne pathogens, including *Salmonella* (Chen 2004; Goldstein and others 2001; Randall and others 2004), *E. coli* O157 (Zhao and others 2001a), *Yersinia enterocolitica* (Soto and others 2003), and *C. jejuni* (Lee and others 2002); commensals (Barlow and others 2004; Hofacre and others 2001; Lu and others 2003; Nandi and others 2004; Roe and others 2003); veterinary pathogens isolated from various animal sources (Bass and others 1999; Goldstein and others 2001; Hudson 2000; Sanchez and others 2002; Schmidt and others 2001); and retail meats (Chen 2004; Roe and others 2003). Their distribution within bacterial populations varies depending on animal source (Goldstein and others 2001), possibly reflecting selection pressures or ecology of each animal niche.

Other mechanisms of genetic exchange

Although conjugation (bacterial cell-to-cell contact allowing transfer of DNA) is probably the most efficient means of antibiotic resistance spread, other mechanisms—transformation and transduction—also play a role. Transformation involves the uptake of naked DNA followed by its subsequent integration into the genome of the bacterial cell. This process is limited in nature to 40 known bacterial species including *Neisseria*, *Acinetobacter*, ε-proteobacteria, *Helicobacter*, *Campylobacter*, *Bacillus*, and select *Streptococcus* species (Lorenz and Wackernagel 1994). Analysis of bacterial genomes, however, suggests that more microorganisms, for example, *E. coli*, *Lactococcus*, and *L. monocytogenes,* may be or were capable of natural transformation earlier

in their evolution (Claverys and Martin 2003). Bauer and others (1999) reported that *E. coli* developed competence and took up free plasmid DNA in model food systems (milk, carrot juice, and soy drink) via transformation. Transformation appears to occur frequently among several members of this select group, evident from the "mosaic" nature of their genomes (Claverys and others 2000). Depending on the microorganism, transformation is limited within the bacterial population, and is influenced by growth phase and restricted in acceptability of donor DNA for uptake (Lorenz and Wackernagel 1994). Restriction–modification systems limit the overall efficiency of transformation for distantly as well as closely related species or strains (Stein and others 1988).

Bacteriophages are also important players in ferrying gene(s) among microorganisms. At low frequency, phages can mistakenly package random bacterial DNA fragments (plasmid or chromosome) and subsequently transmit the genetic information to a new bacterial host. Referred to as generalized transduction, this process may explain dissemination of the *Salmonella* MDR locus of DT104 among *S. enterica* serotypes and strains (Boyd and others 2001; Cloeckaert 2000b; Doublet and others 2003; Meunier and others 2002). Lysogenic[19] phages inadvertently incorporate bacterial DNA flanking their integration site when phage DNA imprecisely excises itself from the bacterial host chromosome. This genetic information is then passed on to the new host cell upon infection and integration of the phage genome into the chromosome. This process of specialized transduction is important in the evolution of both phage and host. Bacteriophages have acquired ancillary genes that encode toxins, lipopolysaccharide modifying enzymes, and other virulence factors (Canchaya and others 2003) as well as antibiotic resistance genes (Muniesa and others 2004). However, the probability at which either process, generalized in contrast to specialized transduction, may occur in nature is influenced by the frequency at which bacterial DNA is inadvertently incorporated into the phage capsid (Sternberg and Maurer 1991), the host range of the phage (Chibani-Chennoufi and others 2004), and inducing host recombination (Sternberg and Maurer 1991).

[19] lysogenic: harboring a prophage as hereditary material (definition from Merriam Webster's Medline Plus: www.nlm.nih.gov/medlineplus/mplusdictionary.com).