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Scientific Status Summary

Antimicrobial Resistance: Challenges and Perspectives

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Abstract: In 2006, the Institute of Food Technologists (IFT) published an Expert Report entitled "Antimicrobial Resistance: Implications for the Food System" (IFT 2006). That report summarized current scientific knowledge pertaining to the public-health impact of antimicrobial use in the food system and the development and control of antimicrobial resistance. Since that time, intense interest in this topic has continued within the regulatory and scientific communities as well as the general public. This IFT Scientific Status Summary serves to update that 2006 IFT Expert Report by briefly reviewing new scientific evidence relevant to the goals of the initial report and providing a number of key observations and conclusions.

Executive Summary

In the time since the publication of the IFT Expert Report, governmental and intergovernmental efforts to address the issue of antimicrobial resistance have seen much progress. In particular, concerns about the public-health implications of microbial resistance to antibiotics used in both human medicine and food-animal agriculture have led to the publication of the World Health Organization's (WHO) List of Critically Important Antimicrobials for Human Medicine and the World Organisation for Animal Health's List of Antimicrobials of Veterinary Importance. The U.S. Food and Drug Administration (FDA), which led with its own tables in 2003, has used such lists as the basis for categorizing various classes of antimicrobials as: important, highly important, and critically important, and has since issued rules that prohibit most extra-label uses of some critically important antimicrobials such as fluoroquinolones and cephalosporins in food animal species. This report concludes that, although more remains to be done to improve the utility of these designations, such categorization of antimicrobials is helpful in prioritizing and addressing public-health concerns and antimicrobial use.

Other effortsto address threats posed by antimicrobial resistance include monitoring programs for antimicrobial-resistant microbes that integrate human, animal, and food sampling schemes. These programs, in various stages of development and implementation worldwide, have been useful in identifying trends in development and persistence of antimicrobial resistance among select foodborne pathogens and related microbes. Two of the most mature such systems, the National Antimicrobial Resistance Monitoring System (NARMS) in the United States and the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) in Denmark, were discussed in the 2006 IFT Expert Report. NARMS, a joint program administered by the FDA, Centers for Disease Control and Prevention (CDC), and United States Department of Agriculture has, during a roughly 14-y period, monitored trends in antimicrobial resistance among foodborne pathogens such as *Salmonella* and *Campylobacter jejuni* that are largely of animal origin. This update discusses developments in the NARMS program since publication of the parent IFT report in 2006. In addition, general observations from NARMS data are presented, including: the lack of resistance expansion to some of the WHO critically important antibiotics, such as fluoroquinolone and quinolone resistance among non-Typhi *Salmonella* serovars; an increase in fluoroquinolone-resistance among *C. jejuni* followed by a sustained level (neither increase nor decrease) after fluoroquinolone use was banned for poultry; very low levels of *C. jejuni* resistance to macrolides; and markedly increasing resistance among *Salmonella* to 3rd generation cephalosporins. Many multidrug resistance (MDR) phenotypes (with or without ceftiofur resistance) among *Salmonella* are serovar-dependent, with the most prevalent being *S.* Newport and *S.* Typhimurium in cattle, and *S.* Heidelberg and *S.* Kentucky in poultry.

Also of note since the publication of the Expert Report in 2006 is substantial interest and questions surrounding the emerging resistant pathogen–methicillin-resistant *Staphylococcus aureus* (MRSA). The increasing incidence of community- and occupational-based infections due to MRSA continues to vex public-health practitioners. In recent years, questions have been raised about the potential for foodborne transmission of this pathogen and the role

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of agricultural antimicrobial use in its emergence. This update discusses pertinent issues and research related to this topic and concludes that, at present, there is little empirical evidence to support its direct relevance to food safety or to infer that antibiotic use in agriculture has played a primary role in its emergence and evolution.

In addition to methicillin resistance in *S. aureus*, increases have been observed in bacterial resistances to multiple antimicrobials since 2006. These increases in co-resistance, that is tolerance to therapeutic concentrations of more than one antibiotic, complicate the challenge of treating drug-resistant bacteria by potentially delaying the selection and application of the most efficacious antibiotics which may result in adverse public-health outcomes. Co-resistance can be conferred by accumulation and genetic linkage of multiple gene cassettes such as that observed on the IncA/C plasmid of *Enterobacteriaceae*. Acquisition of the IncA/C plasmid is of most concern because of the plasmid's ability to acquire large numbers and varieties of determinants conferring drug resistance. This plasmid seems to have a broad host range that enables it to circulate among a variety of bacterial hosts, both commensals and pathogens. One such example of IncA/C plasmid acquisition is by *Salmonella* Newport MDR-AmpC. This serovar is a highly virulent pathogen of special concern because it is not only resistant to ceftriaxone that is the drug of choice for treating salmonellosis in children but is also co-resistant to many alternative drugs.

In addition to antimicrobials, many nonantimicrobial factors such as metals and disinfectants can co-select for antimicrobial resistance in bacteria. Indeed, the use of heavy metals as replacements for antimicrobial growth promotants in Europe, such as elevated levels of feed-grade zinc and copper, have been implicated in helping to expand and sustain MRSA and macrolide- and vancomycin-resistant *Enterococcus* spp., respectively. Pertinent issues surrounding co-resistance and co-selection as well as the implications of these issues for the development of effective interventions are discussed in detail in this report.

Some antimicrobial-resistant bacteria are clearly a foodborne threat (for example, *Salmonella* Typhimurium DT104), whereas others are less likely to be a foodborne hazard (livestock-associated MRSA). Likewise, the role that agricultural antimicrobial selection pressures have played in promoting the emergence and persistence of such strains is perhaps more clear for the former than the latter. Regardless, the issue of co-selection by other contributing factors, including metals and disinfectants, and the serovar-dependence of resistance traits among the *Salmonella* create considerable uncertainty of the magnitude of risk posed by animal and aquaculture uses of these products.

Perhaps because of the complexity and uncertainty associated with co-resistance and co-selection, there is little consensus on what constitutes an effective intervention to mitigate the occurrence of resistance among microorganisms, especially those of public-health relevance. One approach is to reduce or prohibit the use of antimicrobials in agricultural animals. This strategy has been successful for specific "drug-bug-use" combinations, such as when broiler producers in Quebec ceased the *in ovo* administration of ceftiofur. This intervention appears to have contributed to meaningful reductions in the occurrence of ceftiofur-resistant *S.* Heidelberg and *Escherichia coli* on poultry products and in the percentage of ceftiofur-resistant *S.* Heidelberg in human clinical patients. However, in other situations more complex interventions appear to be required. Studies evaluating the Danish experience, wherein non-therapeutic uses of antimicrobials were excluded from livestock production in Denmark, have shown minimal evidence to date suggesting that this intervention has provided substantially greater public-health protection in the form of a reduced disease burden of morbidity and mortality in the human population. Furthermore, the Danish experience illustrates that simple interventions are, in some cases, insufficient to impact resistance levels and associated health outcomes and, further, that metrics of success may be difficult to measure particularly if they are not proximate in terms of time or space to the intervention.

While domestic control over antimicrobial usage policy and monitoring is achievable, little actionable risk management information is available for imported foods. Because of the recognized global concern of antimicrobial resistance in the food supply and the potential for these resistant organisms to be moved around the globe in foods, the Codex Alimentarius Commission (CODEX) established an *ad hoc* Intergovernmental Task Force on Antimicrobial Resistance in 2007. The Task Force had the mandate to provide guidelines for a structured risk analysis framework to address human health risks associated with the presence of antimicrobial-resistant microorganisms in food and animal feed, including aquaculture, and the transmission through food and animal feed of antimicrobial-resistant microorganisms or determinants linked to the nonhuman use of antimicrobial agents. Through this venue, national and regional food safety authorities may soon have increased ability to assess and control risks posed by antimicrobial resistance in imported foods.

Saliently, the prevalence and diversity of resistance to different antimicrobials can vary greatly among countries and regions, and this might be due to substantive differences in antimicrobial usage practices. While we must focus on ensuring that antimicrobial use within the United States is prudent and also continue to discover and evaluate alternatives to antimicrobial use, we also must recognize that food production is internationally interdependent. It is highly likely that actions will be taken during the next 5 y to further restrict the availability of critically important antimicrobials and their allowed uses in aquaculture and agriculture, particularly in the developed world. However, such practices may in the near future have trade implications which will apply pressures to those jurisdictions with less control on their antimicrobial practices to develop and implement appropriate risk management policies. To effectively mitigate harmful effects from antimicrobial resistance in the United States, we must work with global partners to promote prudent use in those countries where regulatory oversight of critically important antimicrobial drugs is underdeveloped.

Introduction

In 2004, the Institute of Food Technologists (IFT) convened a panel of internationally-renowned experts, led by Michael Doyle of the Univ. of Georgia, to address the concern that the use of antimicrobials in production agriculture, food processing, and in human medicine may lead to the emergence, dissemination, propagation, and persistence of foodborne pathogens that are resistant to antimicrobials and unable to subsequently be controlled by them. The IFT panel produced an Expert Report entitled "Antimicrobial Resistance: Implications for the Food System" (IFT 2006). The IFT Expert Report summarized current scientific knowledge pertaining to the public-health impact of antimicrobial use in the food system and the development and control of antimicrobial resistance. Due to the availability of new and pertinent research since the 2006 publication of the Expert Report as well as continuing interest in this topic, this Scientific Status Summary has been prepared to provide an update to the contents of the original report.

Unfortunately, a summary of the entire breadth of new information available is outside the scope of this document. Therefore, this Scientific Status Summary provides a U.S.-centered perspective on 5 key topics of relevance to the antimicrobial resistance issue. These are: (1) the competing views of the criticality of antimicrobials in animal production; (2) trends in antimicrobial resistance as reported by the U.S. National Antimicrobial Resistance Monitoring System (NARMS); (3) relevance of methicillin-resistant *S. aureus* as a foodborne pathogen; (4) importance of antimicrobial co-resistance, co-selection, and interventions in animal production and human therapy, and Denmark's experiences with reducing the use of in-feed antimicrobials; and (5) use of antimicrobials in animal production in various developing countries and the implications for countries importing foods of animal origin from these countries. This Summary provides some general key observations regarding antimicrobial use that were not addressed in the 2006 Expert Report and presents a number of conclusions that can now be drawn on the basis of new scientific information.

Antimicrobial Criticality

Concerns surrounding the public-health impact of the use of antimicrobials in human medicine and food animal agriculture have evolved during the past several decades and have led to development of categorized or prioritized lists of classes of antimicrobials with different focuses, varying regulatory action, and competing views on the criticality of antimicrobials (the importance of protecting antimicrobial treatment efficacy, as it relates to microbial resistance, in medical and veterinary settings). The background on the issue of the criticality of antimicrobials is reviewed below.

Since their introduction into animal agriculture approximately 60 y ago, antibiotics have proven to be highly effective in treating bacterial infections of significant animal health consequence in virtually every food animal species, including poultry and aquaculture (Gustafson and Bowen 1997). In general, there is clear understanding of the mechanisms underlying the action of the antibiotics against targeted bacterial pathogens. For example, bacterial pneumonia, a common problem in recently weaned beef calves, can be prevented, controlled, and treated by using approved drugs from a number of antibiotic classes and in a variety of formulations (Apley 2006). Resistance to antibiotics occurs among the targeted pathogenic bacteria relatively infrequently; even then, when it is measured and then classified using *in vitro* assays, its clinical association has been questioned (McClary and others 2011). The occurrence of resistance among targeted pathogens in food animals is not the issue that raises concern. Instead, it is the unintended consequences of the effects of the antibiotics on the bacteria that often are normally resident in the gastrointestinal tracts of the food animals that raise most public-health and food safety concerns (WHO 2000a,b).

A variety of resistance mechanisms exist, and are generally coded through genes housed on bacteria chromosomes or on mobile genetic elements such as plasmids. These resistance mechanisms have emerged, evolved, propagated, and persisted to permit commensal and pathogenic bacteria that are resident in the gastrointestinal tracts and environments of animals to continue to survive and thrive under the selection pressures exerted by antibiotics (IFT 2006). The "escape" of resistant pathogenic bacteria from the farm into the food supply raises the specter of potentially untreatable infections in human patients that acquire foodborne illness from animal products. It is this sequence of unfortunate events that forms the basis of most contemporary risk assessments in this subject area (Hurd and others 2004; Singer and others 2007). It is important to note that the vast majority of foodborne illnesses caused by bacteria such as *Salmonella enterica* (various non-Typhi serovars), *C. jejuni*, and certain types of *E. coli* are self-limiting and do not require treatment with antibiotics; and, in fact, in some cases use of antibiotics is contraindicated (Wong and others 2000; Safdar and others 2002). It is just as important, however, to point out the imperative that certain antibiotics must remain effective for cases of foodborne illness–particularly those involving invasive bacteria–that warrant therapy. These worst-case scenarios are especially problematic when they involve specific subpopulations of humans, such as children, for whom certain classes of antibiotics might not be approved or may be contraindicated. Examples include the need for sustaining macrolide efficacy for use in treating *Campylobacter* infections, and of 3rd and 4th generation cephalosporins for *Salmonella* infections, in instances in which fluoroquinolone use may be problematic in children (Alghasham and Nahata 2000). Concerns such as these led to recent regulatory actions and the drafting of lists of antimicrobial classes that formally reflect the prioritized need for protecting their efficacy for future use in both human medicine and food animal agriculture. A brief description of the timelines and major developments in codifying the relative importance of antimicrobial classes, both in the United States and around the world, follow.

The World Health Organization's (WHO) advisory group on integrated surveillance of antimicrobial resistance (AGISAR) met in Oslo, Norway in June 2011, to draft the 3rd revision (Table 1) of the "WHO List of Critically Important Antimicrobials for Human Medicine" (WHO 2012). Comprised of practitioners and researchers from human and veterinary medicine and individuals from government, academia, and nongovernmental organizations around the world, the committee's work is merely the latest chapter, and not the final story, of this ongoing and evolving issue. Since development of the 2nd revision in 2009 (WHO 2011), the committee faced several new issues: (1) emergence of Gram-negative enteric bacteria exhibiting extensive multi-drug resistance (MDR) and metallo-beta (β)-lactamase resistance which appeared to have arisen on the Indian subcontinent (NDM-1), (2) the decision by infectious disease physicians to utilize colistin (a longstanding and easily toxic polymyxin available for use in both human medicine and animal agriculture) as a drug of last resort for treating critically ill patients infected with strains that have MDR, and (3) emerging concerns about methicillin-resistant *S. aureus* (MRSA) CC398, *Clostridium difficile*, vancomycin-resistant enterococci, and other "nontraditional" enteric foodborne pathogens.

The formal path toward these lists began at joint meetings of the Food and Agriculture Organization of the United Nations (FAO), World Organisation for Animal Health (OIE), and WHO, held in Geneva in 2003 (FAO/OIE/WHO 2003). At the meetings, it was suggested that both the WHO and the OIE and their members draft listings of antimicrobials of importance to human and veterinary medicine, respectively. The 1st WHO expert meeting on critically important antimicrobials (CIA) was convened almost exclusively with physicians in Canberra, Australia, in 2005. Subsequent to this meeting, the list has been revisited every other year with revisions arising from Copenhagen (2007, 2009), and most recently Oslo (2011). In contrast, the OIE first published its listing of CIA in 2007, which was formally adopted by its general assembly and incorporated into its Terrestrial and Aquatic Animal Health codes (OIE 2007). The OIE listing is scheduled to be revisited in 2012.

While it is difficult to pinpoint exactly where the story of criticality and the importance of each of the various classes of

Table 1–WHO listing (3rd revision, 2012) of critically important antimicrobials for human medicine.

Critically important	Highly important	Important	Unclassified
Aminoglycosides	Amdinopenicillins	Aminocyclitols	Ionophores
Carbapenems and other penems	Amphenicols	Cyclic polypeptides	Bambermycins
Cephalosporins (3rd and 4th generation)*	Cephalosporins (1st and 2nd generation)	Nitrofurantoins	Carbadox
Cyclic esters	Licomsamides	Nitroimidazoles	
Fluoro and other quinolones*	Penicillins (anti-staphylococcal)		
Glycopeptides*	Pleuromutilins		
Glycylcyclines	Riminofenazines		
Lipopeptides	Steroid antibacterials		
Macrolides and ketolides*	Streptogramins		
Monobactams	Sulfonamides		
Oxazolidinones	Sulfones		
Penicillins (natural aminopenicillins and antipseudomonal)	Tetracyclines		
Polymyxins			
Rifamycins			
Tuberculosis and other mycobacterial drugs			

*The top 4 critically important antimicrobials are prioritized based on: (1) high absolute number of people affected by diseases for which the antimicrobial is the sole or one of few alternatives to treat serious human disease, and (2) high frequency of use of the antimicrobial for any indication in human medicine, since usage may favor selection of resistance. In addition, a focusing criterion for the above classifications is that there is a greater degree of confidence that there are nonhuman sources that result in transmission of bacteria or their resistance genes to humans (WHO 2012).

antibiotics began, the Swann report (Swann 1969) is likely the first published report that implicitly considered some antibiotics more important to human medicine than others. Since then, what determines the current importance of an antibiotic can be quite fluid. For example, early antibiotic classes tended to be treated as less important as new synthetic antibiotics were developed and approved (as during the heyday of development of new antibiotics in the 1970s and 1980s). More recently, older classes, perhaps ironically, have once again become more critical as resistance to the newer classes has emerged (for instance, the use of colistin to treat NDM-1 infection) (Bercot and others 2011; Docobo-Perez and others 2012; Poirel and others 2012). As another case in point, the recent rulings in 2011 and 2012 by a district court judge in New York (Katz 2012) which are reflected in pending legislation (PAMTA 2009) have refocused the spotlight on older classes such as tetracyclines and penicillins, while the U.S. FDA has shifted much of its recent focus towards newer classes such as cephalosporins, glycopeptides, and fluoroquinolones (HHS/FDA 2012).

In the United States, the action that most clearly represents a shift in thinking about the criticality of antibiotics deemed important to human medicine is reflected in the FDA decision in 1997 to prohibit the extra-label use of fluoroquinolones and glycopeptides in animal agriculture (HHS/FDA 1997). The law that gave rise to the Animal Medicinal Drug Use Clarification Act ("the AMDUCA rule"), which came into effect in 1994, allowed veterinarians with a valid veterinarian-client-patient relationship to prescribe certain products for use in an extra-label manner. Although some products previously were prohibited for use in animal agriculture (such as chloramphenicol), the 1997 ruling on fluoroquinolones and glycopeptides reflected for the 1st time that resistance development in foodborne bacteria of animal origin was an impetus for the prohibition of extra-label antibiotic use. Previously, most concerns that led to prohibitions on use were driven by concerns over residues and hazards to human health arising from direct exposure to the pharmaceutical products or their metabolites. These prohibitions on animal drug use included (up until May 22, 1997): chloramphenicol, clenbuterol, diethylstilbesterol, dimetridazole (and other nitroimidazoles), furazolidone, nitrofurazone, and sulfonamide drugs in lactating dairy cattle (except approved uses). None of these prohibitions was made on the basis of microbial safety; instead the 1997 decision by FDA represented a turning point whereby bacterial resistance as the sole endpoint (HHS/FDA 1997) was a legitimate reason for prohibiting extra-label uses of animal drugs. The FDA had proposed in 1977 to prohibit in-feed uses of penicillin and tetracycline based on resistance concerns; however, action was never taken and this remains the subject of litigation to the present date (Katz 2012).

With the FDA publication of its Guidance for Industry (GFI #152)–Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to their Microbiological Effects on Bacteria of Human Health Concern–in 2003, a qualitative risk assessment approach was provided as a nonbinding recommendation to industry pursuing preapproval safety evaluation of a new animal drug as part of a new animal drug application process. Among the steps involved, which map closely to the OIE approach to risk assessment (Murray 2004) and depart from the older National Academy of Sciences—National Research Council model (Dean and Scott 2005), hazard identification is extracted from the phases of risk assessment representing a separate and equal module of risk analysis. Then, the steps involved regarding risk from resistant enteric bacteria arising in the food production system would present, in turn, a release assessment, exposure assessment, consequence assessment, and overall risk estimation (FDA 2003; Murray 2004). Because the approach is qualitative, the matrices that were developed are a combination of axes that reflect, generally, 3-point scales of low, medium, and high (release probability, exposure probability, consequence, and overall risk) (FDA 2003).

The consequence assessment is the component that reflects the need to categorize the importance of the antimicrobial class being considered. Indeed, Table A1 (potential ranking of antimicrobial drugs/drug classes based on the identified relevant factors) in the GFI 152 document (FDA 2003) holds the origins of much of the logic and even the text language of subsequent lists, such as those published by the WHO and the OIE. Regarding observed differences between the WHO lists and the GFI 152 Table A1, it is prudent to note that the FDA document focuses on the foodborne pathogen as the hazard, whereas the WHO list includes all zoonotic pathogens, whether foodborne or not, as hazards. The table A1 categorizes the consequence of resistance to various classes of antimicrobials as important (I), highly important (H), and critically important (C) and provides rationale for such classifications. Because GFI 152 is an assessment of risks associated with food safety, it is important to note that the relative importance refers to the use of the class in human medicine rather than animal agriculture or veterinary medicine. Examples for each category

include: (1) important: 1st and 2nd generation cephalosporins; (2) highly important: aminoglycosides, penicillins, glycopeptides, and tetracyclines; and (3) critically important: 3rd generation cephalosporins, fluoroquinolones, and macrolides (FDA 2003). Note that this list has not been updated since 2003; however, any prospective sponsor of a new animal drug application would be expected to refer to contemporary evidence of importance. Some critics have pointed out that no class of antibiotic is less than important to human medicine. However, that is not entirely true. Several classes that are not deemed to be important for human medicine are excluded from the process. These have included (until now, at least) ionophores, bambermycins, and carbadox (WHO 2012). The FDA (2003) list of antimicrobials not deemed important to human health for food safety purposes also includes nonpotentiated sulfonamides and polypeptides (bacitracin); however, these are classified as highly important and important, respectively, in the WHO list (WHO 2012).

In July 2008, the FDA issued a final rule, while seeking comment, prohibiting all extra-label uses of cephalosporins in food animal species (HHS/FDA 2008). Many in the animal health community were surprised and angered when the FDA first issued the "final rule;" and the resultant backlash and substantive comments likely contributed to delays in the final implementation of this order for almost 4 y. The original order was likely facilitated by data originating in Canada concerning *in ovo* injection of ceftiofur at low doses into eggs at broiler hatcheries (Dutil and others 2010). An FDA survey conducted in the United States in 2004 also found that the practice was widely employed in broiler hatcheries (HHS/FDA 2012). The data from Quebec revealed a marked increase in *Salmonella* Heidelberg isolates resistant to ceftiofur and ampicillin and a precipitous decrease in resistance when the practice was voluntarily ceased (Dutil and others 2010). However, since the ruling did not apply simply to that practice, the response to the request for comments was overwhelming and the rule changed substantially from the 1st draft. Because of the overwhelmingly negative and large-scale response to that order, in November 2008 the FDA indefinitely delayed the implementation of the original order to study the hundreds of comments it received (HHS/FDA 2012). In the end, as a result of the critical importance of 3rd and 4th generation cephalosporins and concerns about the misuse of the products in certain commodities, the final order and rule that came into place in April 2012 effectively prohibits any deviation from the label other than for disease indication or exemptions for minor use in minor species. Thus, the order effectively bans cephalosporins for *in ovo* egg injections, "bio-bullets," and self-compounded products such as oral preparations which are the practices that gave rise to concerns in the first place.

Recent outbreak reports have begun to add the abbreviation MDR (multidrug resistance) to the mainstream media vernacular. Outbreaks involving pathogens exhibiting MDR are especially disconcerting when they involve highly pathogenic bacteria such as certain serovars of *S. enterica*. However, caution should be used regarding broad characterizations of a microbial isolate as having MDR if the isolate is simply shown to be resistant to 3 or more classes of antibiotics. The *Salmonella* Heidelberg strain involved in one extended outbreak (or 2 depending on how counted) during the summer of 2011 was triply resistant to sulfa drugs, streptomycin, and tetracycline. Each of these antibiotics is a long-standing product from a different class that would be unlikely to be chosen as initial therapy in human medicine for salmonellosis, should treatment be indicated (Aslam and others 2012; Folster and others 2012a). Other outbreak strains of *Salmonella* Heidelberg have in the recent past harbored resistance to only 2 antibiotics–ampicillin and ceftriaxone–and so would not be labeled as having MDR using the above criteria; however, because 3rd and 4th generation cephalosporins are some of the limited number of highly effective products for treating invasive salmonellosis, especially in children, this resistance profile could easily represent a greater threat than the MDR profile described earlier.

This example illustrates an essential aspect to understanding the threats posed by antibiotic resistance: that a policy of adhering to steadfast rules and categorizations with respect to risks and threats to public health has weaknesses. Keeping a focus on what is most important for protecting human health is a good first step. For example, the various lists of critically important antibiotics, such as those published by the WHO and the OIE and which are revisited and updated regularly, allow inclusion of new antibiotics in higher orders of importance based on new and changing information. However, it is also important to move beyond simple classifications and examine instead what really impacts public health in terms of tangible and measurable outcomes. Where interventions are broadly enacted, are there likely to be predictable and measurable reductions in risk that result? While it can be exceedingly difficult to monitor risk reduction in a tangible manner, quantitative risk assessments with a focus on the numbers of cases (morbidity and mortality), case prognoses, and tangible quality-of-life measures (such as disability adjusted life years, or DALYs) afford some tangible estimates of expected cost/benefit as contrasted to simple invocations of the precautionary principle.

The National Antimicrobial Resistance Monitoring System

In the time since the publication of the 2006 Expert Report, NARMS underwent review by the FDA Scientific Advisory Board's External Subcommittee. The subcommittee found much to commend about NARMS and offered several valuable criticisms. The outcome of the review and subsequent changes to the NARMS program is summarized here. In addition, trends in resistance since 2006 as evidenced in NARMS data are briefly discussed.

An External Subcommittee of the FDA's Science Advisory Board released its review of NARMS in 2007 (FDA/SAB 2007). The subcommittee was able to review 10 y of clinical data from the human and animal/slaughter components of NARMS as well as 5 y of data from the retail meat component. The subcommittee's report found that NARMS was an invaluable asset to the food safety of the nation, declaring that NARMS had become a "mission-critical tool for FDA." Importantly, the subcommittee along with broader stakeholders at NARMS public meetings also offered substantive and largely meritorious criticisms of the program. The subcommittee specifically noted that problems with sampling bias in all program arms impeded external validity of results at the national level and was vulnerable to prior treatment bias in diagnostic samples.

In response to these concerns the analysis and reporting of isolates arising from veterinary diagnostic laboratories was discontinued in the animal arm of NARMS (FDA 2011). While this action alleviates the original concerns raised by the subcommittee and others, subsequent commentary has noted that such diagnostic laboratory isolates could be early indicators of emerging resistance patterns of concern (WHO 2012).

In the NARMS human arm, similar concerns of bias due to a sole reliance on human clinical isolates were noted. However,

because results from human clinical isolates are expected to represent the "tip of the iceberg" and reflect the end of the food-chain, ongoing surveillance in this area was considered important to continue. To improve accuracy of resulting estimates, the committee recommended that sampling be extended to incorporate microorganisms from healthy individuals (FDA/SAB 2007). The subcommittee also suggested that appropriate technology and assays be used to allow for detection of emerging resistance in addition to relying on prevalence estimates from more established resistances. To date, the progress made against this latter recommendation in the human arm remains unclear and is presumed to be progressing slowly, perhaps because such approaches would require substantial changes in current NARMS sample handling and processing procedures. While lack of representative sampling was the primary criticism (both on a national scale, and with regard to microorganisms from healthy individuals who are unlikely to have had prior antimicrobial treatment) the subcommittee also advocated for a strong and flexible system to allow for collaborative and hypothesis-driven research (FDA/SAB 2007).

Another area of concern raised by the subcommittee was in public access to NARMS data, specifically the timing of reporting to the public and allowing 3rd-party, real-time, web-based generation of reports. To this end, the animal arm has created a very useful web-based query tool that allows for aggregated querying on a "bug-drug" (microorganism-antimicrobial) basis (NARMS Animal Arm USDA Interactive Data Query Page 2011). As of 2012, however, the tool does not include the capacity to search for specific multidrug phenotypes or specific genotypes.

Additionally, the subcommittee suggested that the realm of NARMS be expanded globally and that training be extended to and promoted in other countries. This has been accomplished through participation of many of the NARMS principal scientists in the WHO Global Food Network (GFN) for *Salmonella* surveillance, as well as involvement of at least 3 NARMS representatives on the WHO-AGISAR committee (WHO 2012).

During the 5 y since the IFT and FDA subcommittee reports were published, several major trends have emerged and others have continued. Several items of interest are addressed below. Readers are encouraged to seek out each of the current NARMS reports (encompassing animal, human, and retail meat isolates), which are generally cumulative and allow for comparisons with past results and assessment of trends over time (FDA 2012; National Antimicrobial Resistance Monitoring System (NARMS)—Reports 2012). It is also noteworthy that as of August, 2012, several pilot projects exploring the themes of on-farm sampling were underway to determine: sources of resistance variation occurring during preharvest, utility of sampling and recording of antibiotic usage, and the relationship of these approaches to harvest and postharvest venues.

One of the major trends to emerge from continued sampling during a roughly 12-y period is the lack of expansion of resistance to several of the WHO list of critically important antibiotics among specific pathogens (FDA 2012). Of the top 4 antibiotic classes on this list (Table 1), resistance to 2 of these, namely quinolones and macrolides, has remained low or failed to increase in certain important segments of the sampled microflora. Alternately, resistance to 3rd generation cephalosporins appears to be increasing in some, though not all, types of isolates.

Among *Salmonella* isolates, quinolone resistance shows varying trends depending on isolate serovar and origin. Resistance to quinolones, including fluoroquinolones, has remained static in human, animal and retail meat samples for all combined non-Typhi *Salmonella* serovars since the inception of NARMS in 1998. Among these serovars, both quinolone and fluoroquinolone resistance remained negligible; for specific pathogens of concern (such as *S.* Enteritidis, *S.* Typhimurium, *S.* Newport, and *S.* Heidelberg), ciprofloxacin resistance was at or near zero (0.2%, 0.0%, 0.0%, and 0.0%, respectively) (FDA 2012).

Resistance levels have increased in exclusively human *Salmonella* serovars, such as *S.* Typhi and S. Paratyphi, which are largely associated with international travel (FDA 2012). While resistance to the important fluoroquinolone–ciprofloxacin–has remained low for *S.* Typhi and *S.* Paratyphi (2.7% and none, respectively, in human isolates as of 2010), increasing resistance to nalidixic acid has been observed. Levels of this resistance in human isolates of *S.* Typhi increased to 69.1% in 2010. In *S.* Paratyphi, 90.4% of 2010 isolates were resistant. This is of much concern because nalidixic acid resistance serves as a bellwether for fluoroquinolone resistance development in *Salmonella*.

Based on these trends, it is expected that any increase in resistance to antibiotics among human serotypes of *Salmonella* (such as Typhi) is likely to be attributed to human instead of animal antibiotic use. This conclusion stems from the following NARMS data trends discussed above: (1) quinolone, including fluoroquinolone, resistance is low or absent in non-Typhi *Salmonella* serovars, which are associated with both animal and human hosts, and (2) nalidixic acid resistance is increasing in *S.* Typhi and *S.* Paratyphi, serovars exclusively associated with human hosts. The extremely low levels of resistance to fluoroquinolones among *Salmonella* of animal origin remain a relative success in the United States. In all 3 animal hosts (cattle, broilers, and swine), there is virtually no detectable resistance using NARMS sampling protocols. This is not to say that resistance does not occur, but rather that levels are below the detection limit of NARMS. In other countries, especially in the developing world, fluoroquinolone resistance is high and increasing among *Salmonella*, *Campylobacter,* and other bacteria.

Similarly, NARMS data indicate that quinolone resistance and, in particular, fluoroquinolone resistance, in *C. jejuni* has remained stable during the period 2006 to 2010. These data suggest that fluoroquinolone-resistance levels have not diminished following the 2005 U.S. bans on their use in poultry (FDA 2012). However, fluoroquinolone resistance has also not increased substantially since 2005 (as compared to other areas of the world), and remains established at about 22% for both quinolone and fluoroquinolones among human, animal, and retailed meat isolates.

Resistance to macrolide antibiotics, which are also found among the top 4 critically important antibiotic classes as defined by WHO, remains at very low levels in *C. jejuni* (1.5% resistance to both azithromycin and erythromycin among human isolates, 4.0% resistance among poultry isolates in the animal arm, and 0.6% among retail meat isolates). This low level of macrolide resistance could be attributable to the more complex resistance mechanism against macrolides as opposed to quinolones in *Campylobacter* that may render macrolide resistance less likely to occur.

Unfortunately, the situation for 3rd generation cephalosporins is less encouraging than for the 2 WHO critically important classes of antibiotics discussed above (fluoroquinolones and macrolides) (FDA 2012). The levels of resistance to drugs such as ceftiofur (for animals) and ceftriaxone (for humans) appear to be increasing among the Gram-negative pathogens such as *Salmonella* and commensal bacteria such as *E. coli*. However, this is not uniformly the case. For example, prevalence of ceftiofur resistance in *S. enterica* isolates from swine during the period 1997 to 2010 appears to be relatively stable between 1% and 6% (Figure 2) (FDA 2011).

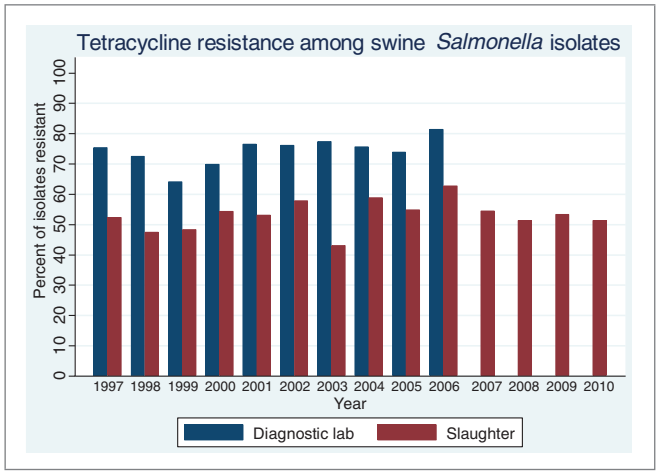

Figure 1–Tetracycline resistance among *Salmonella enterica* isolated from swine samples obtained at slaughter (1998–2010) by the USDA Food Safety and Inspection Service (FSIS) (red bars) and U.S. diagnostic laboratories (1998–2007) (blue bars), and submitted to the National Antimicrobial Resistance Monitoring System (NARMS) for phenotypic analysis. Adapted from 2008 NARMS data (FDA 2011; NARMS Animal Arm USDA Interactive Data Query Page 2011).

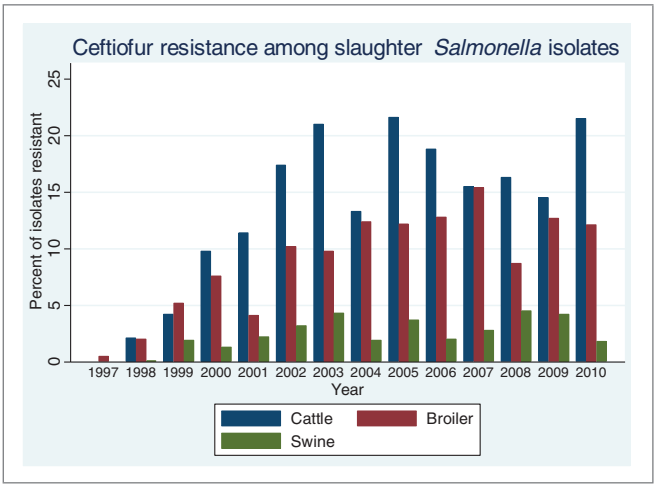

Figure 2–Ceftiofur resistance among *Salmonella enterica* isolated from animal slaughter samples for cattle (blue bars: cannot discern beef from dairy), broilers (red bars), and pigs (green bars) obtained at slaughter (1998–2010) by the USDA Food Safety and Inspection Service (FSIS), and submitted to the National Antimicrobial Resistance Monitoring System (NARMS) for phenotypic analysis. Adapted from 2008 NARMS data (FDA 2011; NARMS Animal Arm USDA Interactive Data Query Page 2011).

Further interpretation of data must be made with care, particularly across serovars of *Salmonella*. Two events that complicate interpretation of these data are: (1) revision of the Clinical and Laboratory Standards Institute's breakpoint for resistance to ceftriaxone in 2010 from 64 to 4 ug/mL, making interpretation much more consistent with ceftiofur resistance (breakpoint of 8 ug/mL); and (2) the occurrence of predominantly plasmid-mediated, single-gene resistance in the United States in contrast to extended spectrum β-lactamases (ESBL) in addition to AmpC and cephamycinases such as $bla_{\text{CMY-2}}$ that have been prevalent in Europe and much of the rest of the world. It is noted, however, that the U.S. situation changed recently (Wittum and others 2010; Mollenkopf and others 2012), and it is likely that ESBLs, such as in strains having CTX-M β-lactamase activity, will become more prevalent in the U.S. food supply in the near future.

Several trends seen in the NARMS data are highlighted here for swine and pork products. First, in terms of evaluating the trends in macrolide resistance (number 3 on the WHO top 3 list because of erythromycin's role in treating campylobacteriosis in children), there is little information because *Campylobacter* is only tracked in NARMS for poultry and not for swine isolates. For swine isolates, as for turkey isolates, the dominant *Campylobacter* species tends to be *Campylobacter coli* rather than *C. jejuni;* and, worldwide, *C. coli* tends to exhibit higher macrolide resistance than *C. jejuni*. In terms of *S. enterica* in swine, information adapted from the NARMS Animal Arm USDA Interactive Data Query Page (2011) and Table 23 of the 2010 NARMS Executive Report (FDA 2011) is shown in Figures 1 and 2. Trends in tetracycline resistance (Figure 1) are used to illustrate the point that tetracycline resistance is highest among isolates of *Salmonella* from diagnostic laboratories. Note, however, that diagnostic laboratory data collection for NARMS ceased in 2007 in response to criticism of the inherent biases in diagnostic data. The tetracycline resistance trends should not be surprising because these isolates are much more likely to be from animals receiving prior antibiotic treatment. Second, although tetracycline resistance is high, it has been essentially stable during the past 14 y. This is in stark contrast to ceftiofur resistance, which is low in swine compared with broilers and cattle (Figure 2) but is increasing. This antibiotic class is number 2 on the WHO list, following fluoroquinolones. The difference in levels of resistance may relate to levels of historical use of ceftiofur in cattle and broilers versus swine. However, it is just as likely to be lower in swine as a simple function of dominant *Salmonella* serovars (Table 2). It is well recognized that many resistance phenotypes among *Salmonella* are serovar-dependent (a trend not seen with nontyped [generic] *E. coli*), with the most prevalent resistant serovar of *Salmonella* in cattle being Newport, whereas in broilers the dominant serovars are Kentucky and Heidelberg. Among pigs, *S.* Derby and *S.* Infantis have relatively low levels of resistance to ceftiofur.

Methicillin-Resistant *S. aureus*

In recent years considerable coverage has been devoted to the increasing incidence of community-based human infections due to methicillin-resistant *S. aureus* (MRSA) and a much more modest number of nonnosocomial (not hospital-based) infections linked to direct occupational and other exposure to food animals. It is important that one distinguish between colonization by MRSA (which can be either transient or well-established) and infection. *S. aureus* has been for a very long time a normal inhabitant (as well as an opportunistic pathogen) of human and animal skin and mucosal surfaces. The organism has evolved to continue to thrive in the presence of selection pressures such as antibiotics. Linkages to virulence factors compound the problem but do not override the fact that MRSA and MSSA (methicillin-susceptible *S. aureus*) can be found on the skin and in the nasal passages of otherwise healthy individuals.

Recent concerns about a specific large-animal *S. aureus* strain, referred to as clonal complex or sequence type 398 (CC398 or ST398) have led to a plethora of well-designed, prospective research studies and publications on this topic (Mevius and Verbrugh 2006; Wagenaar and others 2007; Wagenaar and others 2009; Graveland and others 2010; Mulders and others 2010; Broens and others 2011; Davies and others 2011; Tulinski and others 2012). Other research, however, has been based on simple withdrawals and further study of isolates (of clinical, food animal, and retail

Table 2–Top 10 *Salmonella enterica* serovars isolated from cattle, broilers, and pigs at slaughter in 2008. Adapted from 2008 NARMS data (FDA 2011; NARMS Animal Arm USDA Interactive Data Query Page 2011).

Cattle	Nr of Samples	%	Broiler	Nr of samples	%	Pigs	Nr of samples	%
Montevideo	104	23.5	Kentucky*	219	35.1	Derby	25	22.5
Newport*	53	12.0	Enteritidis	116	18.6	Infantis	15	13.5
Dublin*	31	7.0	Heidelberg*	94	15.1	Agona*	6	5.4
Anatum	27	6.1	Typhimurium v 5-*	39	6.3	London	6	5.4
Cerro	27	6.1	Typhimurium*	31	5.0	Saintpaul	6	5.4
Typhimurium*	25	5.6	I 4,[5],12:i:-	23	3.7	Typhimurium v 5-	6	5.4
Kentucky	22	5.0	Infantis	14	2.2	Anatum*	5	4.5
Muenster	18	4.1	Montevideo	13	2.1	Johannesburg	5	4.5
Agona*	17	3.8	Schwarzengrund	7	1.1	Ohio	4	3.6
Representing:	**324**	**73.1**	**Representing:**	**556**	**89.1**	**Representing:**	**78**	**70.3**
Out of:	**443**	**100**	**Out of:**	**624**	**100**	**Out of:**	**111**	**100**

*Moderate to strong association with ceftiofur resistance.

meat origin during the past several decades) from existing freezer banks. Isolate-based, as opposed to sample-based, research overlooks the importance of formalized random sampling from the population of hosts and their bacteria in the analysis. Decades-old isolate banks have the potential to be dominated by isolates cultured from clinical human patients, or completely lacking in similar nonclinical sampling schemes. More recent isolate stocks include broadly derived isolates from clinical patients as well as hypothesis-driven field research and active and passive surveillance representing nonclinical animal and retail-meat sampling. Taken as whole, inference made on such disparate isolate sources introduces "selection bias" as described, for example, by Dohoo and others (2009).

The European Food Safety Authority (EFSA) quietly put the issue as a food safety concern to rest in 2009 (EFSA 2009). The EFSA scientific opinion of the Panel on Biological Hazards (2009) noted that: "There is currently no evidence for increased risk of human colonisation or infection following contact or consumption of food contaminated by [MRSA] CC398 both in the community and in hospital. MRSA (including CC398) can enter the slaughterhouse in or on animals and occurs on raw meat. Although it may become part of the endemic flora of the slaughterhouse, the risk of infection to slaughterhouse workers and persons handling meat appears to be low based on currently available data." The Panel (EFSA 2009) also noted that: "Where [MRSA] CC398 prevalence is high in food-producing animals, people in direct contact with these live animals (especially farmers and veterinarians, and their families) are at risk of colonization and subsequent infection. The potential for CC398-colonized humans to contribute to the spread of MRSA in hospitals currently seem to be less than for hospital associated MRSA strains." However, other ongoing research projects and genomics analyses of existing isolates from clinical human, retail meat, and healthy animals collected for many years, with no account given to selection bias, have led to some arguably overstated inference concerning the roles that animal agriculture may have played in expanding the resistance and virulence repertoire of this specific subtype of *S. aureus* (Price and others 2012).

Davies (2012) prepared a thorough and thoughtful summary of the current state of knowledge on MRSA CC398. In brief, currently the major concern regarding this strain is that of nosocomial transmission (for example, biosecurity for those patients entering hospitals that have robust infection control, surveillance, and quarantine/cohorting measures, such as occurs in The Netherlands). While not discounting the roles that any *S. aureus* can have in an opportunistic infection, it appears from disease reporting to the U.S. CDC that *S. aureus* CC398 is rarely present in cases of either community-based or nosocomial origin in the United States (Davies and Linhares 2012). In short, little empirical evidence exists to support the relevance of MRSA to food safety, or to permit legitimate inference regarding the role that animal agriculture and antibiotic use in particular might have played in its emergence and evolution.

Co-resistance, Co-selection, and Interventions (for example, the Denmark Experience)

Co-resistance (the tolerance to therapeutic concentrations of more than one class of antimicrobial) substantially complicates the challenge of drug resistance among pathogenic and commensal bacteria and is a major concern of the public-health community worldwide. Co-resistance to classes of drugs that are the preferred therapies for the treatment of bacterial infections limits therapeutic options and can result in treatment delays with the most efficacious drugs which can result in adverse public-health outcomes. In some instances, bacteria are resistant to several drug classes–the phenomenon frequently referred to as MDR. The generally accepted definition of MDR is co-resistance to 3 or more classes of antimicrobial drugs. As explained above, however, this may be an overly simplistic definition that does not take into account the criticality of the drug classes to which the bacterium is resistant.

Low-level, nonspecific MDR can be conferred by nonspecific efflux pumps such as those encoded by the multiple antibiotic resistance (MAR) operon. Alternatively, drug-specific co-resistance can be conferred by the accumulation of various gene cassettes, such as that observed in *Salmonella* Typhimurium DT104 (Threlfall 2000; Ribot and others 2002). This globally disseminated bacterium displays a well-described, penta-resistant phenotype which is typically co-resistant to ampicillin, chloramphenicol, streptomycin, sulfa drugs, and tetracyclines (ACSSuT MDR phenotype). In this instance, the gene cassettes responsible for the phenotype are housed in a type 1 integron, located in the bacterial chromosome.

More recently, an even broader and decidedly more troublesome co-resistance phenotype has emerged and has primarily, but not exclusively, been observed in *S.* Newport (Fey and others 2000; Zansky and others 2002; Zhao and others 2003a). In addition to the ACSSuT MDR phenotype described above, resistance to 3rd generation cephalosporins and potentiated β-lactams has also been observed, and is generally conferred by the $bla_{\text{CMY-2}}$ gene cassette (Zhao and others 2003a; Alali and others 2009). In contrast to the genes encoding the ACSSuT phenotype, the $bla_{\text{CMY-2}}$ cassette does not appear to be housed within the type 1 integron, and most bacteria harbor multiple copies of this gene. Further, the genetic material responsible for this so-called MDR-AmpC phenotype

(that is, ACSSuT plus resistance to a 3rd generation cephalosporin and potentiated β-lactams) is typically housed on a large mobile (or mobilizable) IncA/C plasmid (Carattoli and others 2002; Carattoli and others 2006; Poole and Crippen 2009; Poole and others 2009). It is also possible that this AmpC-β-lactamase gene can be housed on other plasmids such as the IncI1 plasmid observed in *S.* Heidelberg (Folster and others 2012b).

The IncA/C plasmid appears to have a particularly broad host range, having been identified in a wide variety of environmental and animal-sources, including bacteria recovered from fish (McIntosh and others 2008; Verner-Jeffreys and others 2009), production animals (Zhao and others 2001; Daniels and others 2007; Frye and Fedorka-Cray 2007; Evershed and others 2009), companion animals, humans (Evershed and others 2009; Marcarde and others 2009; Sirichote and others 2010; Veldman and others 2010), water (Pan and others 2008; Verner-Jeffreys and others 2009), and soil. The emergence of the IncA/C plasmid and its variants among these populations and ecological niches is of concern because of its: ability to acquire large numbers and varieties of drug-resistant-encoding mobile genetic elements (Fricke and others 2009; Call and others 2010; Toleman and Walsh 2010), broad host range which enables it to circulate among a variety of environmental and commensal bacterial hosts and niches (Suzuki and others 2010), and ability to be transferred among both commensal and pathogenic bacterial species (Poole and others 2009). *E. coli* and *Salmonella* that harbor IncA/C plasmids have been routinely recovered from food-producing animals and meat in North America (Welch and others 2007; Zaidi and others 2008). The mobile potential of the IncA/C plasmid may help to explain why the MDR-AmpC phenotype has been observed among an increasingly broad variety of *Salmonella* serovars and other *Enterobacteriaceae* (Lowrance and others 2007; Kunze and others 2008; Platt and others 2008).

Most salmonellosis illnesses are self-limiting and do not require antimicrobial therapy. When invasive salmonellosis occurs in children, ceftriaxone, a 3rd generation cephalosporin, is the drug of choice (Fey and others 2000). Consequently, *S.* Newport MDR-AmpC, a highly virulent serovar, poses a particular challenge because it is resistant not only to the drug of choice for children but also is co-resistant to many potential alternatives. While not yet resistant to fluoroquinolones in North America, this class of antibiotics is contraindicated for use in treating salmonellosis in nonadults, further limiting the choices. In addition to the emergence of co-resistance among foodborne pathogens, of concern within health-care settings is the emergence of resistance among various other bacteria such as Gram-positive cocci or other *Enterobacteriaceae* that are opportunistic pathogens which can cause nosocomial infections.

Co-resistance may also result in co-selection or co-amplification of the resistant bacteria and plasmids. For instance, if a bacterium is co-resistant to drugs A and B, the ostensibly logical hypothesis is that the use of drug A will co-select for resistance to drug B and *vice versa*. This concept is most easily understood from a laboratory pure culture viewpoint but is more complex in a host, which typically has many and varied bacteria (such as within the gastrointestinal tracts of animals or humans). For example, it was observed that the use of ceftiofur in cattle both co-selected for and facilitated co-amplification of nontype-specific *E. coli* that were co-resistant to tetracycline as well as other classes of drugs (Lowrance and others 2007; Alali and others 2009). This finding would suggest that the administration of tetracycline in cattle would also select for ceftiofur resistance. However, the authors of a study of cattle observed that, paradoxically, ceftiofur-resistant *E. coli* were less likely to be recovered during the period of time in which tetracycline was administered in the feed (Platt and others 2008).

The authors of the latter study speculate that due to differences in relative fitness among bacteria, even within a species, the use of an antimicrobial drug may select for the fittest bacterial subpopulation. Hence, if the fittest ceftiofur-resistant *E. coli* are also co-resistant to tetracycline, then the use of ceftiofur would co-select for tetracycline resistance, as has been observed. Conversely, however, if bacteria that are singly resistant to tetracycline were fitter than those co-resistant to tetracycline and ceftiofur, then tetracycline use would primarily favor the singly resistant *E. coli* rather than preferentially co-select for the *E. coli* that were resistant to both ceftiofur and tetracycline. Hence, it is hypothetically possible that tetracycline use may have actually provided a competitive disadvantage for ceftiofur-resistant bacteria in that specific cattle population (Platt and others 2008). This example illustrates the sometimes highly complex nature of co-resistance mechanisms and indicates that researchers must carefully consider the role of complex and competitive bacterial interactions. Study designs and analyses should target the elucidation of true causal mechanisms. Without this type of careful and thoughtful research, the development of effective interventions to control resistance will be severely impeded.

It is often speculated that drug-resistant bacteria, such as the *Salmonella* serovars exhibiting MDR, emerge or are propagated in production animal agriculture and that the use of antibiotics in animals contributes to this emergence and propagation (Angulo and others 2000; Fey and others 2000; Angulo and others 2004a, b). While this may be true, there are surprisingly few empirical data that substantiate that speculation. A recent and salient analysis of phenotypic resistance patterns in a historical database of *Salmonella* Typhimurium DT104 recovered from animals and people in Scotland provides interesting insights, albeit regionally- and bacteria-specific, into the directionality of resistance spread (Mather and others 2012). In their analysis, the number of phenotypes obtained from both animals and people was observed. It is important to note that the breadth of phenotype diversity was valued more than depth of any particular phenotype, and the 2 sources (diagnostic laboratory samples from human and animal clinical cases) likely introduced unmeasured selection biases into the analysis. That said, however, overall the resistant phenotypes tended to be detected 1st in the human population and, moreover, the diversity of phenotypes was greatest within this population. This does not imply that bacteria from food animals do not pass to humans. Clearly bacteria from food animals do reach, infect, and sometimes cause disease in people via food, as a result of direct contact with animals, or indirectly from the environment–but the study does highlight that emergence and diversity of resistance in co-resident populations of animals and people is complex and does not represent a simple unidirectional pathway from animals to people. It may also be inferred from the analysis by Mather and others (2012) that *S.* Typhimurium DT104 might be considered a quasi-clone that is evolving both independently and interdependently in humans and cattle. That is, there may be macro- (interdependent) and micro- (independent) ecological drivers that influence the bacterium's evolution. This highlights the possibility that factors unrelated to antimicrobial exposure may play a role in the co-evolution, co-emergence, and persistence of bacterial resistance phenotypes, both in this specific system (Scotland) and more broadly.

In fact, there are many nonantimicrobial factors such as metals and disinfectants that can potentially co-select for antimicrobial resistance (Summers 2002). Use of any of these co-selectors could further select for and facilitate persistence of an antibiotic resistance gene. Once introduced into specific environments, the persistence of antibiotic resistance genes could be due to the presence of additional genes that confer resistance to chemicals and metals which provide an ecological fitness advantage to the bacteria (Alonso and others 2001). For example, a plasmid frequently found in avian pathogenic *E. coli* (Johnson and others 2006) possesses genes that confer resistance to tetracycline, streptomycin, gentamicin, sulfisoxazole, copper sulfate, and benzylkonium chloride (a quaternary ammonium compound), among others. In one study, isolates that exhibited MDR were significantly more likely ($P < 0.01$) to harbor copper resistance than other isolates, suggesting a genetic linkage between multiple resistance mechanisms (Johnson and Nolan 2009).

The discussion above illustrates the complexity of resistance dynamics and demonstrates how difficult it can be to pinpoint the precise causes of co-resistance emergence, spread, and persistence. Thus, although the desire and need to intervene are self-evident, there is little consensus on what constitutes an effective and practical intervention. Since antimicrobial use provides a selective pressure that favors bacteria that can tolerate the concentration of antibiotic at the site of colonization, a straightforward approach is to reduce or prohibit the use of antimicrobial drugs. This intervention strategy has seen some success. In Quebec, Canada, for example, producers voluntarily ceased the *in ovo* administration of ceftiofur (Dutil and others 2010). Meaningful reductions were observed in the percentage of ceftiofur-resistant *E. coli* and *S.* Heidelberg isolates recovered from poultry and the percentage of ceftiofur-resistant *S.* Heidelberg isolates from human clinical patients. This indicates that in some unique drug-use-bacteria combinations, a simple intervention can be quite effective.

However, such simplistic interventions have not been able to control resistance in other situations. In Denmark, amongst other efforts to control the spread of resistance growth-promotion claims were removed from antibiotics, leading to effective and dramatic reduction of in-feed use of antimicrobial drugs, according to the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) (Aarestrup and others 2010). One of the primary desired outcomes of this intervention was to protect public health; yet, there is little evidence to suggest that this was achieved. For instance, there are few, if any, reports that demonstrate reductions in the burden of resistance in bacteria recovered from ill individuals or that treatment success has improved. It may well be that changes in public-health metrics following implementation of an intervention take years to appreciably change and may be difficult to document, at least in a manner that is proximate to the intervention.

For some bacterial species and antibiotics there may be some evidence of success. Resistance to vancomycin and quinupristin/dalfopristin in various bacteria from broilers and pigs is extraordinarily rare in Denmark. Furthermore, some evidence indicates reductions in salinomycin and tetracycline resistance in *E. faecium* recovered from broilers and pigs, respectively (DANMAP 2012). Yet in other bacteria such as *Salmonella* or *Campylobacter* there is little evidence to suggest that Denmark has experienced meaningful improvements in decreasing the antimicrobial resistance (Aarestrup and others 2010; DANMAP 2011, 2012). For example, the percentage of *Salmonella* Typhimurium that are resistant to ampicillin, tetracycline, and/or a sulfonamide has continued to increase since the intervention was introduced. Furthermore, the proportion of *C. jejuni* isolates resistant to nalidixic acid and/or erythromycin has similarly increased. While it may be possible that the rate of increase is slower than what might have occurred had the ban not been implemented, there is little evidence to indicate that the Danish intervention reduced the reservoir of antibiotic-resistant *Campylobacter* and *Salmonella* in food animals. This may indicate that some bacteria (for example, pathogens such as *S.* Typhimurium) are able to amplify and spread without antimicrobial selection pressure. Therefore, simply removing antimicrobials from the system may not be sufficient to address resistance in certain organisms or systems. In addition, the choice of indicator organisms to judge the success of national intervention strategies may not be straightforward.

While the intervention in Denmark did dramatically decrease the use of in-feed antimicrobials, it also increased the use of various drugs, including cephalosporins, for therapeutic purposes (Aarestrup and others 2010). It is possible, therefore, that the increased therapeutic administration of specific antimicrobial drugs contributed to the persistence and real increase in the phenotypic resistance that has been observed since the Danish intervention was introduced. The authors of one study concluded that swine productivity, when analyzed as mean number of pigs per sow per year raised for slaughter and average daily weight gain, increased since the intervention was introduced (Aarestrup and others 2010). It would ostensibly appear that Danish producers are more productive without "production" uses of antibiotics. However, the number of swine producers in Denmark has decreased from approximately 25000 in 1995 to less than 10000 in 2005. During this time, the Danish pork industry has become more integrated and intensive, and now consists of larger producers. It is plausible that the producers with the highest productivity and efficiency survived and grew in size. This trend has occurred around the developed world as well, so it is difficult to associate the ecological effects of industry consolidation and antimicrobial cessation with the productivity of any single producer. Nevertheless, it is probable that the producers who left the industry were the least efficient and productive. Consequently, estimates of overall productivity, such as those reported, are confounded by the changes in production dynamics of the Danish pork industry. Unfortunately, information about specific producers and their productivity over time is not available; therefore, it is not possible to determine how much of an increase in productivity occurred on an individual producer basis. If such information were available, a more rigorous analysis that included approaches to control bias could be performed to better understand the real impact–if any–that the national intervention had on productivity.

The Danish experience highlights how difficult it is to gauge the success or failure of interventions on desired outcomes–even those most proximate to the intervention. Soon, The Netherlands will embark on an intervention to reduce the use of antimicrobials in animal production by setting targets/limits on the defined daily doses (DDD) administered. While it is unclear how the targeted DDD will correlate with public-health outcomes, The Netherlands' approach will provide another opportunity to evaluate a national strategy to control resistance of foodborne pathogens in animal populations.

What is clear is that national or regional intervention efforts that seek to control the impacts of antibiotic resistance on human and animal health should be based on an understanding of the important pressures that can co-select antibiotic resistance (Singer and others 2006). Without taking these co-selectors into account,

interventions will likely fail to produce the desired outcomes and may cloud our understanding of the value, or lack thereof, of intervention.

Implications of Importing Foods

Antimicrobial resistance is a global concern. The prevalence and diversity of resistance to different antimicrobials can vary greatly among countries and regions, and is likely due in part to dramatic differences in antimicrobial usage practices. For example, a recent study of antimicrobial resistance of *Salmonella* isolates from chickens in China revealed that *Salmonella* Indiana and *S.* Enteritidis were the dominant serotypes, with ≥60% of the *S.* Indiana isolates resistant to enrofloxacin, norfloxacin, and gentamicin, many isolates resistant to 16 antimicrobials, and ≥65% of *S.* Enteritidis isolates resistant to ampicillin, tetracycline, and doxycycline. The authors attributed the high rate of antimicrobial resistance to extensive and unwarranted use of antibiotics to prevent and control bacterial contamination of poultry (Lu and others 2011).

Because of the recognized global concern of antimicrobial resistance among microbial contaminants in the food supply and the potential for these resistant microorganisms to be moved around the globe in foods, in 2007 the Codex Alimentarius Commission established an *ad hoc* Intergovernmental Task Force on Antimicrobial Resistance. The Task Force's mandate ("terms of reference") was "To develop guidance on methodology and processes for risk assessment, its application to the antimicrobials used in human and veterinary medicine as provided by FAO/WHO through JEMRA, and in close cooperation with OIE, with subsequent consideration of risk management options. In this process work undertaken in this field at national, regional and international levels should be taken into account." As stated in Principle 4 of the adopted final Guidelines document, "Foodborne [antimicrobial resistance] risk analysis should consider national and regional differences in the use of antimicrobial agents, human exposure to and prevalence of foodborne [antimicrobial resistance] microorganisms and determinants, as well as available risk management options (RMOs)" (CAC 2011). It should be expected that countries and regions will utilize these risk analysis guidelines to establish risk management options that might include the need to limit imports from certain countries because of antimicrobial usage practices and/or antimicrobial resistance patterns in specific bacteria. These restrictions, however, would have to be based on a demonstrated elevated risk to human health using the risk analysis guidelines and after assessing various risk management options.

In some instances, some countries are able to use antibiotics that are not currently approved for use in food-producing animals in other countries or use antibiotics imprudently, leading to widespread contamination in the surrounding environment. In other instances, low costs of animal feed and labor in some countries allow flexibility in export supply chains. Animals can be raised without any antibiotics and sold in an antibiotic-free supply chain, but if these animals get sick and need antibiotic treatment, the meat can quickly be diverted into a different, nonantibiotic-free supply chain.

Fish and seafood are already largely produced outside of the United States. Approximately 86% of fish and seafood consumed in the United States in 2010 was imported (NOAA 2011). This included more than 1.1 billion pounds of shrimp and more than 360 million pounds of tilapia that were largely produced by aquaculture in Southeast Asia. Thailand, Indonesia, China, and Vietnam are primary producers of shrimp (Globefish 2012) and China is the major exporter of tilapia, providing 70% of total U.S. imports in 2009 (USDA/ERS 2012). Both shrimp and tilapia are frequently grown in Southeast Asian countries in small 1- to 2-acre ponds, and are fed raw animal manure (such as poultry or swine feces) as their primary source of direct or indirect nutrients (Little and Edwards 2003; Adewumi and others 2011). Foodborne pathogens such as *Salmonella* are frequently associated with animal manure (Koonse and others 2005) and studies have revealed that 8.5% of imported raw crustaceans (of which more than 93% of the samples was raw shrimp) were contaminated with *Salmonella* compared with 3.9% of domestic raw crustaceans (Heinitz and others 2000).

Table 3–Antibiotic contaminants from Vietnamese shrimp farming.

Antibiotic	Location in shrimp pond	Concentration
Oxolinic acid (Fluoroquinoline)	Surface layer	10 to 25000 μg/L
	Bottom layer	10 to 2310 μg/L
	Sediment	1.8 to 426 mg/kg
Norfloxacin (Fluoroquinoline)	Surface layer	60 to 6060 μg/L
	Bottom layer	84 to 4040 μg/L
	Sediment	6.5 to 2616 mg/kg
Sulfamethoxazole	Surface layer	40 to 2390 μg/L
	Bottom layer	40 to 5570 μg/L
	Sediment	4.8 to 820 mg/kg

From Tuan and Munekage (2004).

A major challenge to growing seafood and fish under such conditions is economic loss due to bacterial diseases caused by *Vibrio*, *Pseudomonas*, and *Aeromonas* species. A common practice to control such bacterial diseases is to treat ponds with antibiotics; frequently, antibiotics deemed critically important, such as fluoroquinolones, are used. For example, ciprofloxacin and oxytetracycline are used extensively (almost 100%) in Vietnamese shrimp farming to kill shrimp disease-causing bacteria during shrimp larvae rearing (Thuy and others 2011). Most Vietnamese farmers use antibiotics in shrimp pond management prophylactically; some do so on a daily basis, and at least 30 different antibiotics are used (Holmoström and others 2003).

Antibiotics used widely in shrimp rearing result in high levels of residues in shrimp ponds as well as the surrounding environment. Examples of antibiotic contaminants observed in Vietnamese shrimp ponds are shown in Table 3. A relatively high prevalence of bacteria resistant to these antibiotics, particularly those used at concentrations of 0.1 μg/mL, was observed in most of the sites studied (Le and others 2005). *Bacillus* spp. and *Vibrio* spp. were predominant among the resistant bacteria. Studies by Zhao and others (2003b) revealed that antimicrobial-resistant *Salmonella* are present in a variety of seafoods imported into the United States, including shrimp from Vietnam. A study of 120 uncooked seafoods (including shrimp, sea bass, oysters, and blood cockels) purchased at retail in Thailand revealed that 26 (22%) were contaminated with *Salmonella* of which 31% of the isolates were resistant to chloramphenicol, 46% to ampicillin, and 42% to tetracycline (Woodring and others 2012). Thailand is the largest shrimp exporter to the United States, accounting for more than 35% of all shrimp imported in 2010 (FAO 2010).

While we must focus on ensuring that antimicrobial use within the United States is prudent and also continue to discover and evaluate alternatives to antimicrobial use, we must recognize that food production is internationally interdependent. Further, we should work with countries where regulatory oversight of critically important antimicrobial drugs is underdeveloped.

Key Observations

Antimicrobial drug use favors bacteria that can tolerate the concentration of a drug present within specific sites of animals,

(such as the gastrointestinal tract) and those that are less susceptible and sometimes resistant to the drug administered. Further, co-resistance to therapeutic concentrations of more than one class of antimicrobials greatly increases the challenge of controlling drug-resistant bacteria.

There have been many relevant findings and significant contributions to the antimicrobial resistance literature, understanding of antimicrobial resistance, and public debate since publication of the IFT Expert Report in 2006. These include:

- The various lists of critically important antibiotics, such as those published by WHO and OIE, are a good first step for focusing on what is most important for protecting public health. Subsequent steps will be needed and might include international collaboration to better understand appropriate science-based regulatory oversight and enforcement to meaningfully protect these critically important drugs.
- Caution should be used in relying on the broad characterization of foodborne pathogens as multi-drug-resistant, as this classification alone may not represent a major threat to public health if the component resistance traits are not considered to be of "critical importance" according to WHO or FDA.
- The U.S. National Antibiotic Resistance Monitoring System (NARMS) has become a mature and respected system for monitoring changes in antimicrobial resistance in human, animal, and retailed meat sources.
- Several noteworthy findings regarding antimicrobial-resistant microbes have been recorded by NARMS during the past 5 to 12 y, including:
  - Among specific pathogens, resistance to several of the top antibiotic classes on the WHO list of critically important antibiotics has not expanded. For other drug-bug combinations, resistance increases were noted. For example:
    - Fluoroquinolone and quinolone resistance has remained static among non-Typhi *Salmonella* serovars. Among exclusively human serovars of *Salmonella* Typhi and *S.* Paratyphi that are largely associated with international travel there is a high level of resistance to nalidixic acid, the bellwether of fluoroquinolone resistance.
    - The prevalence of fluoroquinolone resistance of *C. jejuni* has remained static at about 22% following bans on its use in poultry. In contrast, other countries in the developing world have seen massive expansion of resistance to this class of antimicrobial.
    - Resistance to 3rd generation cephalosporins has increased for some serovars of *Salmonella*.
    - Many antimicrobial resistance phenotypes are serovar-dependent among *Salmonella*, with *S.* Newport being the most prevalent resistant serovar in cattle and *S.* Kentucky and *S.* Heidelberg the most prevalent serovars in broilers.
- Data available thus far fail to implicate MRSA as a foodborne pathogen.
- The emergence of the IncA/C plasmid in bacteria associated with land- and aquatic-grown animals is of concern because of the plasmid's ability to acquire large numbers of varieties of drug-resistant-encoding genetic elements and its ability to circulate among a wide variety of bacterial hosts, including commensals and pathogens such as *Salmonella*.
- *In vitro* models of co-selection for co-resistant bacteria in pure cultures are overly simplistic. In competitive, mixed-culture environments such as the gastrointestinal tract, differences in relative fitness among bacteria make co-selection quite complex.
- A broader understanding of the factors that co-select for antimicrobial resistance is needed as is an understanding of those factors that may either: (a) favor colonization by broadly susceptible commensal bacteria, and/or (b) provide a competitive disadvantage for broadly-resistant bacteria.
- Emergence of antimicrobial resistance in bacteria associated with co-resident populations of animals and people is a complex issue and does not represent a simple unidirectional pathway from animals to human individuals.
- The elimination of growth promotion claims from antimicrobial labels in Denmark, often termed the Danish experience, has not resulted in clear improvements in various antimicrobial resistance or health metrics. This may highlight the challenges in detecting and reporting benefits of regional interventions.
- While simple interventions have been sufficient to control the prevalence of resistant bacteria in some unique drug-use-bacteria combinations, many situations call for more complex interventions.
- A national intervention program to reduce antimicrobial-resistant bacteria should be based on an understanding of the key factors that can select and co-select for antibiotic resistance.
- There is a growing awareness that the regulatory oversight of antibiotics—particularly those deemed critically important—is underdeveloped in some countries and could pose challenges to efforts aimed at mitigating harm associated with antimicrobial resistance.
- While we must focus on ensuring that antimicrobial use within the United States is prudent and also continue to discover and evaluate alternatives to antimicrobial use, we must recognize that food production is internationally interdependent. A more global approach to addressing antimicrobial resistance is needed.

Conclusions

Various international and national agencies and stakeholder groups have developed lists that categorize classes of antimicrobials as critically important, highly important, or important to the continued success of medical and veterinary practice. In the United States, regulatory actions in the form of prohibitions on certain extra-label use of cephalosporins and fluoroquinolones, which are critically important antibiotics, have resulted from such risk classifications. Such categorization of antimicrobials is helpful in prioritizing and addressing public-health concerns and antimicrobial use.

Monitoring programs for antimicrobial-resistant microbes that integrate human, animal, and food sampling schemes are in various stages of development and implementation worldwide, with mature systems having been sustained for over 15 y in countries such as Denmark and the United States, among others. Such monitoring programs have been useful in identifying trends in development and persistence of antimicrobial resistance among select foodborne pathogens and related microbes.

Some antimicrobial-resistant bacteria are clearly a foodborne threat (such as *Salmonella* Typhimurium DT104), whereas others are less likely to be a foodborne hazard (livestock-associated MRSA). Likewise, the role that agricultural antimicrobial selection pressures have played in promoting the emergence and persistence of such strains is perhaps more obvious for the former than the latter. Regardless, the issue of co-selection by other contributing factors, including metals and disinfectants, and the serovar dependence of resistance traits among the *Salmonellae* create considerable

uncertainty of the magnitude of risk posed by animal and aquaculture uses of these products.

While domestic control over antimicrobial usage policy and monitoring is achievable, little information or actionable risk management information is available for imported foods. This may change in the near future through venues such as CODEX.

It is highly likely that actions will be taken during the next 5 y to further restrict the availability of critically important antimicrobials and their allowed uses in aquaculture and agriculture, particularly in the developed world. However, such practices may in the near future have trade implications which will apply pressures to those jurisdictions with less control on their antimicrobial practices to develop and implement appropriate risk management policies.

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