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THE ROLE OF ANTIBIOTICS IN AGRICULTURE

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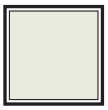

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Recognizing Scientific Excellence

The Role of Antibiotics in Agriculture

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IV

Executive Summary

The development of antibiotics has provided much success against infectious diseases in animals and humans. But the intensive and extensive use of antibiotics over the years has resulted in the emergence of drug-resistant bacterial pathogens. The existence of a reservoir(s) of antibiotic resistant bacteria and antibiotic resistance genes in an interactive environment of animals, plants, and humans provides the opportunity for further transfer and dissemination of antibiotic resistance. The emergence of antibiotic resistant bacteria has created growing concern about its impact on animal and human health.

To specifically address the impact of antibiotic resistance resulting from the use of antibiotics in agriculture, the American Academy of Microbiology convened a colloquium, "Antibiotic Resistance and the Role of Antimicrobials in Agriculture: A Critical Scientific Assessment," in Santa Fe, New Mexico, November 2-4, 2001. Colloquium participants included academic, industrial, and government researchers with a wide range of expertise, including veterinary medicine, microbiology, food science, pharmacology, and ecology. These scientists were asked to provide their expert opinions on the current status of antibiotic usage and antibiotic resistance, current research information, and provide recommendations for future research needs. The research areas to be addressed were roughly categorized under the following areas:

- Origins and reservoirs of resistance;
- Transfer of resistance;
- Overcoming/modulating resistance by altering usage; and
- Interrupting transfer of resistance.

The consensus of colloquium participants was that the evaluation of antibiotic usage and its impact were complex and subject to much speculation and polarization. Part of the complexity stems from the diverse array of animals and production practices for food animal production. The overwhelming consensus was that any use of antibiotics creates the possibility for the development of antibiotic resistance, and that there already exist pools of antibiotic resistance genes and antibiotic resistant bacteria. Much discussion revolved around the measurement of antibiotic usage, the measurement of antibiotic resistance, and the ability to evaluate the impact of various types of usage (animal, human) on overall antibiotic resistance. Additionally, many participants identified commensal bacteria as having a possible role in the continuance of antibiotic resistance as reservoirs. Participants agreed that many of the research questions could not be answered completely because of their complexity and the need for better technologies. The concept of the "smoking gun" to indicate that a specific animal source was important in the emergence of certain antibiotic resistant pathogens was discussed, and it was agreed that ascribing ultimate responsibility is likely to be impossible. There was agreement that expanded and more improved surveillance would add to current knowledge. Science-based risk assessments would provide better direction in the future.

As far as preventive or intervention activities, colloquium participants reiterated the need for judicious/prudent use guidelines. Yet they also emphasized the need for better dissemination and incorporation by end-users. It is essential that there are studies to measure the impact of educational efforts on antibiotic usage. Other recommendations included alternatives to antibiotics, such as commonly mentioned vaccines and probiotics. There also was an emphasis on management or production practices that might decrease the need for antibiotics. Participants also stressed the need to train new researchers and to interest students in postdoctoral work, through training grants, periodic workshops, and comprehensive conferences. This would provide the expertise needed to address these difficult issues in the future. Finally, the participants noted that scientific societies and professional organizations should play a pivotal role in providing technical advice, distilling and disseminating information to scientists, media, and consumers, and in increasing the visibility and funding for these important issues.

The overall conclusion is that antibiotic resistance remains a complex issue with no simple answers. This reinforces the messages from other meetings. The recommendations from this colloquium provide some insightful directions for future research and action.

INTRODUCTION

Antibiotic agents have been used since their development with much success against infectious diseases. In addition to being used in humans and companion animals, antibiotics have been used extensively in agriculture for many years for multiple purposes. Antibiotics are used as growth promoters for food animal production and for therapeutic and prophylactic uses in humans, animals, and plants. The intensive and extensive use of antibiotic agents, however, has resulted in the emergence of highly drug-resistant bacterial pathogens. Some of these pathogens are resistant to most commercially available antibiotics.

The extensive use of antibiotics in humans, animals, and plants has resulted in establishment of a pool of antibiotic resistance genes in the environment. Non-pathogenic and pathogenic organisms that become resistant to antibiotics may serve as reservoirs for resistance genes. This may provide the opportunity for exposure of naive populations of animals, humans, and bacteria to antibiotic resistance genes and antibiotic resistant bacteria and the opportunity for transfer of those genes. Research studies have shown the transmission of pathogenic organisms and possibly antibiotic resistant bacteria/genes from animals through food, water, and by direct contact to humans. The transmissibility of antibiotic resistant bacteria or antibiotic resistance genes among animals and humans or transfer of genes from antibiotic resistant bacteria to otherwise naive bacteria associated with animals underscores concerns about the use of antibiotics in agriculture. The concerns are three-fold: (1) that antibiotic resistance genes are amplified in the environment because of antibiotic use in agriculture; (2) that these antibiotic resistance genes negatively impact public health; and (3) that antibiotic resistance genes negatively impact animal health and production.

Growing concerns about antibiotic resistance have led professionals to organize numerous meetings and forums in an attempt to develop priorities, strategies, and directions for research and education. The primary focus for most of these meetings has been human medicine and public health. The objective of this colloquium was to provide an opportunity to focus on the impact of antibiotic resistant bacteria in agriculture and to provide a forum for a critical assessment of that impact. The short-term goal was to capture expert opinion on the best approaches to understanding and investigating antibiotic resistance, transfer of antibiotic resistance genes, and intervention strategies to prevent the selection and spread of antibiotic resistance genes. The long-term goal was to develop comprehensive approaches for research into this area and to develop possible strategies to help reduce antibiotic resistance.

Participants in the colloquium were asked to provide information on the current status of antibiotic usage and current policies governing their uses. Participants also were asked to provide opinions and directions for new research. The research issues were roughly divided into four areas: (1) origins and reservoirs of antibiotic resistance, (2) transfer of antibiotic resistance, (3) overcoming/modulating antibiotic resistance by altering usage, and (4) interrupting transfer of antibiotic resistance.

CURRENT STATUS

The natural inclination is to equate the amount of antibiotics used in human and veterinary medicine to the amount of existing antibiotic resistance; however, the relationship between use and antibiotic resistance is not linear and seems to be much more convoluted. This issue is complex, particularly in agriculture, because food animal production is multifarious, is the result of diverse management practices and production goals, and each system employs distinct antibiotic use by producers, veterinarians, and others (NAS, 1999).

COMPLEXITY OF PRODUCTION

With the heterogeneity of food animal production comes a complex array of management and production practices dependent on the distinct animal species. For example, beef stocker calves, after being raised for a short time on grass, are shipped to feedlots containing thousands of commingled animals from many distant sources in order to achieve market weight. Conversely, dairy cattle can be housed in large or small herds, but each cow is milked individually. Swine production can be a farrow-to-finish continuous flow operation (i.e., care of an animal from birth to slaughter) or can be an age-segregated multi-site "all in, all out" management system. The poultry industry is highly integrated where a few companies control the majority of all aspects of production. Integration has resulted in standardization of management practices, treatment, and drug usage. Despite integration, there are some differences in management practices and drug usage among chicken breeders, layers and broilers, and turkey production because each is raised for a distinct purpose and has unique requirements. Aquaculture provides other unique production challenges. There are many species of fish and shellfish, each with different husbandry and nutritional requirements. Fish may be raised in open systems, raceways, cages or nets, earthen ponds, closed systems, and bag-and-rack systems, to name a few. For example, open and raceway (series of troughs) systems use adjacent waters or fast flowing streams, respectively, while closed systems control water quality in holding tanks. The majority of finfish are raised in a wide variety of types and sizes of ponds.

USE OF ANTIBIOTICS

The various ways of producing food from animals creates contrasting rates of infection, disease, and, consequently, disparate antibiotic use (Prescott, et al., 2000). For example, the herd density of feedlot cattle can result in viral or bacterial infections that are induced by stress-related factors. Antibiotics are most often given in the feed to help prevent infections or stress-related diseases. They also may be used at different dosages (usually lower) to help promote faster growth. Conversely for dairy cattle, treatment or prevention of mastitis can be by administration of antibiotics by local intra-mammary infusion or by systemic injectable antibiotics. Poultry generally are given antibiotics in feed or water since individual treatment is impractical and not economical; this method of dispensing antibiotics exposes all the animals to antibiotics, but the individual dose is unknown and inconsistent.

There are only two antibiotics approved in the United States for some types of aquaculture, and these usually are given in feed. The fact that these animals are raised in water provides a unique environment for study. The enteric flora of aquaculture is reflective of the water and feed. If fish are raised in warm waters contaminated with commensals or human pathogens, then there are more opportunities for the two-way transfer and dissemination of antibiotic resistant bacteria and antibiotic resistance genes. Fish raised in clean, cold water have relatively low microbial loads and, consequently, are less likely to carry human pathogens.

In U.S. plant agriculture, antibiotic usage is limited to streptomycin and oxytetracyline, which are used as prophylactics for major plant diseases, such as Erwinia. Resistance is widespread to streptomycin and non-existent in oxytetracyline; therefore, oxytetracycline use has increased. The volume and extent of these uses appears relatively minor compared to other agricultural applications of antibiotic agents (Vidaver, 2002).

Colloquium participants agreed that use of antibiotics in companion animals is an essential component to address, although it was not appropriate for this forum. Use of antibiotics

for companion animals is important because antibiotic use may add to the pool of antibiotic resistance genes/bacteria in the environment. These antibiotic-resistant bacteria may be transferred to other animals or humans. There have been several reports of similar bacteria in both humans and animals (Besser, et al., 2000; Deming, et al., 1987). It also is logical that if bacteria can be transmitted from other animals, such as cattle, to humans, then bacteria could be transmitted from companion animals to humans (Fey, et al., 2000).

Another complexity identified is the classification of antibiotic use. There is no standardized terminology of categories (NAS, 1999). Some reports have divided antibiotics into therapeutic and non-therapeutic (or subtherapeutic) categories. Other reports have divided antibiotics into therapeutic, growth promotion, and prophylactic (or preventative) use. The terms *therapeutic, growth promotion,* and *prophylactic* have specific definitions, but if the terms *non-therapeutic* or *subtherapeutic* are used, the definitions are more likely to be interpreted differently by various individuals and organizations. Standardization of the definition of drug use is essential for further research studies, development of judicious use guidelines, and for comparability. Multiple claims on the label of one antibiotic (e.g., growth promotion, prevention) make the differentiation of use more difficult.

The amount of antibiotics used also is subject to confusion. Recent studies by the Animal Health Institute (AHI) and the Union of Concerned Scientists (UCS) illustrated the variation and disagreement of the categorization of drugs (Mellon, et al, 2001; AHI, 2000). The Union of Concerned Scientists estimates that 24.6 million pounds of antibiotics are used for non-therapeutic use. UCS combined growth promoters and disease preventatives under non-therapeutics and did not measure therapeutic antibiotics. Conversely, AHI estimated that a total of 17.8 million pounds of antibiotics are used for all purposes, based on a survey of member companies. In the AHI study, all antibiotics (therapeutic and non-therapeutic) were measured. In addition, ionophores and arsenicals also were measured, although they are not considered traditional antibiotics. By either accounting, the amount of antibiotics used in agriculture is large. This is not surprising given the enormity of food animal production in the U.S.

MEASURING USAGE

The AHI and UCS studies demonstrate the difficulty in accurately measuring the quantities of antibiotic drug used in food animals. Both groups struggled with measuring the amount of antibiotic used. AHI estimated the total kilograms of active ingredient by class of drug used, while UCS used a series of formulas that calculated the label doses to be administered to animals, number of animals in a particular age category, and estimated the number of animals that might receive that dosage.

Colloquium participants felt that it is necessary to get better estimates of antibiotic usage. A significant barrier in the U.S. to obtaining data on antibiotic use in animals is that not all antibiotics used for animals are by prescription. Much of the drug used is sold over the counter. Thus, use data must be collected from various sources, such as pharmaceutical companies, producers, and veterinarians. No one source is complete, and each has its own inherent bias. For example, pharmaceutical companies can provide the amount of antibiotic sold, but this does not translate into what is actually used. Currently in the U.S. there is no national collection or surveillance system for drug usage information. In other countries, such as Denmark, all drug use is by prescription, quantities used are recorded, collated, and reported (Bager, 2000). Similarly, within the United Kingdom the Veterinary Medicines Directorate (VMD) publishes comprehensive details of antibiotic use in food production animals annually. However, some developing countries have no estimates of drug use because of poor infrastructure and illegal drug importation and use.

Understanding the importance of gathering better data on antibiotic use in food animals, the World Health Organization (WHO) convened a consultation meeting in September 2001 to make recommendations on the monitoring of drug use in food animals (http://www.who.int/emc/diseases/zoo/antimicrobial.html). Recommendations included establishment of national monitoring programs for the total usage of antibiotics in agriculture

and collection of very specific data, such as species, production classes, route of administration, and purpose of use. A similar document has also been published by the Office International des Epizooties (OIE Revue 2001; 20: 3.). The logical assumption is that any use of an antibiotic will create the selective pressure for resistance; therefore, perhaps the total amount used is not as important for developing mitigation strategies as determining what types of use (route, dose, duration) cause the highest increase in antibiotic resistance. This data could lead to intervention strategies and guidelines for judicious use.

U.S. POLICIES AND ACTIVITIES

In 1999, the U.S. Food and Drug Administration (FDA) followed proposed guidelines with a document entitled "A Proposed Framework for Evaluating and Assuring Human Safety of the Microbial Effects of Antimicrobial New Animal Drugs Intended for Use in Food-Producing Animals" (Framework Document, FDA, 1999). This document elucidates strategies for managing risks associated with the use of antibiotics drugs in food-producing animals. Strategies include (1) categorization of antibioitics based on their importance in human medicine; (2) revision of the pre-approval safety assessments for new animal drug applications to assess microbial safety; (3) post-approval monitoring for resistance development; (4) collection of food animal antibiotic use data; and (5) establishment of regulatory thresholds. While the framework document mainly outlines some guidelines, it is likely that parts of all of the recommendations will be adapted (either directly or indirectly) and will be used to guide new product development. Additionally, there are legislative initiatives before the U.S. Congress to adapt strong regulations regarding what antibiotics cannot be used for non-therapeutic uses (i.e., growth promotion and prophylaxis) in animals.

The American Veterinary Medical Association (AVMA) and numerous producer groups have begun to develop judicious use guidelines for prudent use of antibiotics (http://www.avma.org/scienact/jtua/default.asp). Guidelines continue to be developed for each animal species. However, the impact of judicious use guidelines is minimal unless the guidelines target specific effective interventions or mitigations and are disseminated and used by a large number of end-users.

One of the most recent U.S. activities was the development by federal agencies of the U.S. Public Health Plan to Combat Antimicrobial Resistance (http://www.cdc.gov/drugresistance/actionplan/index.htm). This is a blueprint for actions that will reduce antimicrobial resistance that is heavily focused on human and public health, but also contains specific actions and initiatives for agriculture and veterinary medicine (Torrence, 2001).

INTERNATIONAL

The WHO has held several meetings in the last few years on the issues of antibiotic resistance and food animals. Each meeting has produced numerous recommendations for action, surveillance, research, and education (WHO, 1997; WHO, 2000; WHO, 2001). Some policies and actions from other countries may serve as models for U.S. actions. At a minimum, international activities may provide a means of determining the impact of certain interventions or policy strategies on, for example, the

elimination of growth promoters. Studies regarding the benefit of growth promoters were conducted in the 1950s, but few have been conducted recently (IOM, 1989; Jukes, 1986). There are some questions about the efficacy and risk/benefits of growth promoters. In 1986 Sweden banned the use of growth promoters in animal production, and in 1999 Denmark banned their use in swine and in broilers (Wierup, 2001; Emborg H-D, et al., 2001). After the ban, Denmark reported an increase in the therapeutic use of antibiotics to help control disease outbreaks, but overall antibiotic use and antibiotic resistance have been reported to decrease (Aarestrup, et al., 2001). It may still be too early to make a complete assessment. Differences between the U.S. and the U.K. also may indicate possible research areas, e.g., the differences in seasonal patterns for *Campylobacter* prevalence and the emergence of *Salmonella* Typhimurium DT104 (Threlfall, 2000). As already mentioned, in developing countries, the use of antibiotics is difficult to control and to measure its impact.

Research Issues

General Comments

The consensus of colloquium participants was that assessing the impact of antibiotics used in agriculture is very complex and subject to much speculation and strong polarization of views. There was little doubt, however, that antibiotic use in agriculture has contributed to the emerging problem of antibiotic resistance. There was general discussion of the extent to which resistance has become a problem. Most participants agreed that animal producers use antibiotics because they are cost effective. Therefore, studies in "pharmacoeconomics" of antibiotic usage were encouraged. In addition, we suggest that studies be developed to assess and identify quantitatively some outcomes, such as the amount of excess morbidity and mortality due to antibiotic resistance resulting from antibiotic use in agriculture (or medicine) or specific causes for treatment failures in humans. Also, studies that measure specific consequences of on-farm use of antibiotics on the emergence of specific resistances are essential.

It also is considered pertinent to determine whether antibiotic usage led to increased susceptibility to secondary infections, particularly by antibiotic resistant pathogens, and whether pathogen load was increased regardless of whether disease occurred. One question raised is whether resistant organisms tended to be more virulent. However, all participants quickly recognized that attempts to determine the importance of any one component in the selection of antibiotic resistant pathogens is almost an impossible task and that specific research tools to assess specific contributions may not be available. It was clear among the participants that any usage of antibiotics would influence the selection of microbes that are resistant to antibiotics. In spite of the many limitations of methods and interpretations of data, it was generally agreed that further research would lead to some relevant answers to this emerging problem. We also recognize that studies involving risk assessments and surveillance must be part of the research portfolio.

The concept of judicious or prudent use of antibiotics in agriculture was voiced repeatedly.

However, it is clear that even defining judicious or prudent use is difficult because of the variety of diverse use practices and reasons for usage among the different groups of animals. One message that did emerge was that for judicious/prudent use practices to be effective, broad acceptance of the practices would be required, and the means of antibiotic distribution in the U.S. needed to change. In particular, the practice of over-the-counter sales of antibiotics in the animal production industry is not conducive to judicious/prudent use practices. The judicious use of antibiotics is likely to extend the useful life of current and future antibiotics by using these drugs only when they are warranted. This obviously is important in preserving the usefulness of antibiotics used to treat humans with life threatening infections and in preserving the usefulness of antibiotics for animals. This is an essential step since the number of new compounds likely to be developed for animal health purposes will be less than for human usage. This is particularly true, in light of the regulatory and political initiatives, the current actions in the European Union (EU), and the lack of economic incentives for the pharmaceutical industry.

ORIGINS AND RESERVOIRS OF RESISTANCE

It was generally agreed that the specific evolutionary origin of resistance genes is unknown. It is likely that many of these genes were derived from antibiotic-producing microorganisms or by other microorganisms that live in proximity to antibiotic-producing microbes. The important issue is where the antibiotic resistance gene reservoirs were developed, maintained, and amplified and the extent to which bacteria in animals or their environments are important reservoirs of these genes. In this regard, origin also could be defined as its first emergence in pathogenic populations of bacteria. Using that definition and given the question of the importance of antibiotic usage in agriculture, tracing back to the initial source or finding the "smoking gun" is important, but very difficult for most current antibiotics. This is because of the already wide distribution of resistance genes and because most drugs are used in multiple animal species including humans.

Likewise, development of complex genetic elements encoding resistance, like the penta-resistance present in *Salmonella* Typhimurium DT104, is likely to have occurred in a stepwise fashion that could have resulted in different animal and human populations. That stated, colloquium participants agreed that for existing drugs, it no longer is important whether the initial resistance gene was selected in human or animal populations, but it is very important what each contributed to the maintenance and/or amplification of resistance gene reservoirs. Furthermore, the likelihood of being able to determine the site of resistance gene selection probably could not be determined with certainty utilizing currently available technologies.

Much discussion revolved around the different ways to measure resistance and which was most accurate. This could be done using methods to detect an antibiotic resistance phenotype or a genotype. There was considerable discussion regarding phenotype versus genotype in the context of pathogens and commensal microbes. If the question of measuring antibiotic resistance is in a pathogen, it was agreed that the measurement should be phenotypic using an inhibition-based assay, such as Kirby-Bauer or broth dilution techniques. There was no consensus on the specific methods that should be used to measure phenotype, although use of National Committee for Clinical Laboratory Standards (NCCLS) standards was a unifying choice (NCCLS, 2002). Yet NCCLS standards for antibiotic resistance in many animal pathogens have not been established.

It was noted that some antibiotic resistance is not based on specific antibiotic resistance genes, but due to physiologic changes rendering the bacterium insensitive to the antibiotic. There also was discussion of the relevance of minimal inhibitory concentration (MIC) data and whether a more effective standard of measurement was a minimal effective concentration (MEC). Such values would inherently be linked to *in vivo* use and the concentration that led to clinical cures. For commensal organisms that could serve as gene reservoirs, expression of the resistance gene was not viewed as important. Since these organisms are not pathogens (at least in

their normal mammalian habitats), they might not express antibiotic resistance(s) despite carrying antibiotic resistance genes. What is important is whether these antibiotic resistance genes are transmissible. Furthermore, without reference standards (like NCCLS), the relevance of a resistance phenotype has little predictive value. It is the gene that is considered important and whether the gene could be transmitted to other bacteria. Thus, for commensal microbes it is the ability to carry a specific gene(s), rather than phenotypic measurement, that is important. Thus, the term "antibiotic resistance" is partially defined by the microbe, and antibiotic resistance only holds clinical importance if it is related to the outcome of treatment associated with a pathogen.

The extent to which commensal microbes serve as reservoirs of resistance genes is not known. The number of commensal microbes in the intestinal tract of a mammal is immense (estimated on the order of 10^{14}), which means that commensal microbes far outnumber pathogens in the gut. Based on this tremendous numerical differential, colloquium participants agreed that commensal microbes act as important reservoirs and vectors of resistance genes and that further research needs to be performed to determine the extent of their importance (Salyers, 1995). Because of the large diversity of commensal microbes present in animals, and because many anaerobic commensals are difficult to culture, we suggest that specific indicator organisms might be a useful approach for the analysis of reservoir populations. No clear-cut definition of which organism would serve as indicator organisms was established. Whole population-based analyses were proposed as providing the greatest ability to assess resistance gene reservoirs where community DNA from a habitat is assessed for the presence of specific antibiotic resistance genes or mobile genetic elements that are important in the dissemination of antibiotic resistance.

A final consideration in evaluating the origins of antibiotic resistance is the need to understand the specific selective environment. What are the true selective factors for antibiotic resistance in the environment and in animals? Many plasmids and integrons encoding antibiotic resistance genes also encode resistance to heavy metals or quaternary ammonium compounds that also could be the selective factor. For example, the integrons encoding resistance to antibiotics in *Salmonella enterica* Typhimurium DT104 are complex and any one of the five specific antibiotic resistances or resistance to quaternary ammonium compounds could select for the other linked antibiotic resistances. The role of environmental contamination (such as water) with antibiotics and other selective agents remains unknown and needs to be factored into any equation of selective influences for antibiotic resistance.

TRANSFER OF RESISTANCE

Because the development of antibiotic resistance is an *in vivo* occurrence, studies on the transfer of resistance described in terms of *in vivo* properties are needed. To date, most antibiotic resistance gene transmission studies have been performed in laboratory settings using microbes usually within the same genus. Since transmission of antibiotic resistance genes is likely to occur via a variety of commensal microbes, additional studies demonstrating inter-generic transmission are needed. Equally important are studies that measure transmission between microbes in animal habitats. These studies should include population-based approaches. A major question is the impact of already existing large antibiotic resistance gene reservoirs and the ability to measure and follow transmission. For example, the gene pool of resistance to tetracycline is believed to be well established and widespread, and measurement of transmission dynamics could be hampered. The use of "marked" strains or strains containing reporter genes in antibiotic resistance genes will provide essential tools that could be used to perform these experiments.

The use of studies of naturally naïve populations, e.g., newborns or places where antibiotics are not used, could be of value in defining the size of the antibiotic resistance gene pool before introducing the selection pressure of antibiotic(s) use. Linked to this is the ability to distinguish between transmission and clonal selection. The specific question in this instance is whether the genes are being transmitted or if it is a specific pathogen being

transmitted. We believe that both methods of transmission have occurred and that genetic methods of discriminating between these are important in assessing the degree of resistance transmission.

A helpful methodological tool for studying resistance in populations is surveillance. The development of surveillance tools, development of effective surveillance strategies, and mechanisms to fund surveillance studies are viewed as important components to stem the rise in antibiotic resistance. The establishment of baseline antibiotic resistance levels is essential for any surveillance study. Given the widespread distribution of antibiotic resistant bacteria in animals and the environment, baseline data for most antibiotics will only represent a measurement of the current situation. However, to develop sound tactics to control antibiotic resistance, it is essential to be able to detect changes in antibiotic resistance levels, whether it is an apparent increase or decrease of antibiotic resistance.

The development of risk analysis models and the measurement of the impact of prevention strategies are dependent on good surveillance methods. The current National Antibiotic Resistance Monitoring System (NARMS) program is an important first step in the surveillance of foodborne and animal pathogens. However, we also believe that this program needs expansion to include the entire "farm-to-fork continuum," as well as better representative sampling schemes. In addition, this surveillance needs to be linked to specific focused studies that would create a more proactive system. It was noted that funding for surveillance studies is limited and that surveillance programs are inherently expensive to perform. Limited funding certainly will lead to limited studies. Consequently, any studies that are performed, including the NARMS project, requires statistically valid and appropriate sampling strategies to produce scientifically sound data that will make good use of limited funding. It was noted that a one size fits all approach might not be applicable for all antibiotic resistance, which also complicates study development.

In colloquium deliberations, it was assumed that transmission of antibiotic resistant bacteria and genes flow in both directions between animals and humans (either by direct contact or via indirect mechanisms). However, the extent to which this occurs has not been systematically investigated. It is likely that there are unique mechanisms in transmission and that these differences are specific for each drug, microorganism, and genetic element.

There was universal agreement that one of the essential components needed to assess the impact of using antibiotics in agriculture on the development of antibiotic resistance in pathogens was quantitative risk assessment. We strongly urge that, in making policy decisions, well designed, science-based risk assessment studies be the basis of long-term decisions. We acknowledge that there are many data gaps hindering even the simplest risk assessment analyses and that additional research is needed to fill these gaps. Concern about the amount of time and funding that it

would take to fill these gaps was voiced and whether in some cases the use of "precautionary principle" to limit some usage of antibiotics was warranted. The precautionary principle states that if harm to humans resulting from continual antibiotic use in agriculture is believed to be likely—and of sufficient severity or magnitude—that action to limit its impact can be taken in the absence of a complete scientific analysis. Concern was expressed about the ability to coordinate and communicate the needs for data among the necessary players, as well as achieving cooperation among them. Finally, there was recognition that the risk assessment method in regards to microbial risk assessment was still in its infancy and needed further study, refinement, and expertise.

OVERCOMING/MODULATING RESISTANCE BY ALTERING USAGE

The most certain means to avoid antibiotic resistance is to not use antibiotics. While this probably is not realistic, the development of specific prudent use guidelines is needed. However, a one-size-fits-all approach to all animal species probably is not feasible. Based on the diversity of antibiotics used with different animals species, as well as the purposes for use, any guidelines will be inherently complicated. Yet their existence is needed and their impact can be critical. One universal criticism of antibiotic usage in agriculture in the U.S. is the availability of many antibiotics over the counter. This means that producers of livestock can use antibiotics without the need for a prescription. It is generally thought that veterinarians are trained to be able to make prescribing decisions. Veterinarians have the medical and scientific expertise to make informed decisions about what antibiotic to use and appropriate dosing regimens. While we believe that veterinarians should be consulted more often in production decisions, we also recognize that this might cause an economic burden to the producers. We also recognize that veterinary medical professionals need continuing education about judicious use guidelines. We believe strongly that additional research should be done to determine if more effective dosing regimens could be developed to help minimize the potential for development of antibiotic resistance.

There was considerable discussion of the likelihood of whether the restriction of the use of antibiotics in animals would lessen the burden of antibiotic resistance gene reservoirs. There are minimal data available to describe what would happen in natural experiments where antibiotic usage is eliminated. Results from Sweden and Denmark suggest that restricting or eliminating specific uses does result in reduced carriage of antibiotic resistance genes. However, most of these claims remain preliminary and need to be systematically studied. It was proposed that some consideration of cost versus benefit ratio was necessary, although it might not guide use practices.

On a cellular level, some *in vitro* experiments have shown that carriage of antibiotic resistance genes is accompanied by a decrease in cell fitness. However, the fitness cost can be reduced or eliminated by compensating mutations in the microbe. Since the precise conditions that are selective for maintaining antibiotic resistance genes are not known, and since there could be multiple selective factors, the elimination of one does not necessarily reduce the need to keep the genetic element(s) encoding antibiotic resistance genes. Smith, et al. (2002) propose a model that has been reinterpreted by Lipsitch, et al. (2002) that concludes that once the antibiotic resistance gene is initially selected (in animals), it can be maintained by transmission to bacteria found in humans with no further antibiotic use in that animal population. Knowing whether this model—which states, "Once the cow is out of the barn, closing the door will not get it back"—is valid and universal to all antibiotic resistance genes is of the utmost importance. Answers to this question will guide the veterinary, agricultural, and medical communities in determining whether antibiotics (and which ones) should be withdrawn from agricultural use.

Many of the antibiotics used in agriculture are drugs that were developed in the 1950s and are considered quite old. However, many of these drugs (e.g., tetracycline) apparently retain good biological activities as antibiotics for some pathogens in some species of animals and continue to function as growth promoters. Studies should be performed to determine if they really retain good growth

promotion activities and how they work. In the face of widespread and extensive antibiotic use, there is a question of why they would work. One interpretation is that we may not be measuring the correct drug-induced activity. For example, do antibiotics have other properties that are tied to growth promotion?

In the face of huge antibiotic resistance gene reservoirs to the older drugs, one would expect that the drugs would retain little therapeutic activity. This leads one to question whether *in vitro* susceptibility testing really relates to *in vivo* activities. It is possible that the answer to these and other questions about why some older drugs still work will allow us to develop new antibiotics with certain characteristics and guide us on how current antibiotics should be used.

The use of antibiotics for purposes of growth promotion and disease prevention is thought to be a major component of the antibiotic usage pattern in animal agriculture. Since all uses of antibiotics lead to the cumulative effect in the selection for antibiotic resistance, if the reliance of antibiotics for non-therapeutic purposes could be decreased, there would be an impact on reducing the selective pressures for antibiotic resistance. We strongly recommend that research to develop new probiotics and vaccines is a priority. In addition to these products, pre-harvest management procedures need to be developed that reduce the need for antibiotics and thus lessen the potential for emergence of antibiotic resistance.

INTERRUPTING TRANSFER OF RESISTANCE

The reduction in transmission of antibiotic resistance can be considered at several levels. The most obvious is prevention of transmission and acquisition of the specific resistance genes. There is very little information on how to do this. In fact, without knowing the specific microbial sources, including commensal, of the antibiotic resistance genes that are being transmitted in farm (or other) settings, blocking the process would only be based on empirical measurements. The transmission of certain antibiotic resistance genes is an inducible process (i.e., some genes encoding resistance to tetracycline) where the actual antibiotic is the inducing agent. However, how widespread this phenomenon is, and in which microbial populations, is unknown. An understanding of the transmission of antimicrobial resistance determinants among bacteria, both within species, and across species and genera, also is important. At another level, the question is how to prevent transmission of antibiotic resistant microbes between animal and human populations and into relevant environmental habitats. Also pertinent is the determination of the extent to which transmission of antibiotic resistant bacteria from humans to animals occurs and the extent to which such transfer impacts the efficacy of antibacterial use in human medicine. These questions represent some additional data gaps that are important in developing tactical approaches to reduce antibiotic resistance.

Recommendations

- Better estimates of antibiotic usage are needed. This will involve a system of collecting data with standardized elements definitions. Development of this system may be helped by evaluating current international systems and by considering that over-the-counter use of drugs makes it more difficult to obtain data. There have been suggestions by other organizations that antibiotic drug use be by prescription only. Better usage data will enable properly designed and science-based effective intervention and mitigation strategies.

- Additional studies are needed in pharmacoeconomics. For example, what are the costs and the benefits of antibiotic use? What are the costs and the benefits of the use of growth promoters? What is the cost of alternative management or production practices that might decrease the need for antibiotic use? Economic studies should also be conducted on the outcome of antibiotic use. For example, what is the cost of antibiotic resistance in terms of excess mortality, morbidity, and treatments?

- It is essential that appropriate research studies be developed to evaluate the impact of prevention, intervention, or control strategies on antibiotic resistance.

- Judicious use/prudent guidelines need to be more widely disseminated among end-users and veterinary professionals. More proactive educational efforts are needed. Successful educational programs can help reduce improper antibiotic use, decrease the possibility of antibiotic resistance, and help prolong life of antibiotic. Studies that will evaluate the impact of these programs are vital.

- It is important to determine where gene reservoirs are developed, maintained, or amplified, particularly when the origin of resistance may not be found. This research data will provide useful hypotheses for interventions or control.

- A consensus is needed on the measurement standard of antibiotic resistance, e.g., genotype vs. phenotype. Resistance measurement depends on the specific pathogen or whether the measurement is of a commensal microbe.

- More research is needed on the role of commensals as reservoirs, including anaerobic bacteria. Whole population-based analyses were proposed as providing the greatest ability to assess reservoirs in a community.

- A better understanding of selective factors or pressures on the development of resistance is needed. What is the role of the environment?

- Additional studies are needed on the transfer of resistance in vivo, such as the transmission between microbes in animal habitats. Population-based approaches, the use of naïve populations, marker strains, or strains containing reporter genes might be useful in experimental studies. There is a need to discriminate between clonal and other types of transmission.

- Surveillance (including NARMS) systems exist but need improvements in areas of sampling strategies, funding, and laboratory tools. We need to continue to strive to make the systems responsive to changes in emerging organisms, temporal trends, and the interaction of multiple factors affecting resistance.

- Quantitative risk assessment is an essential scientific tool in evaluating antibiotic resistance and in providing information for policy and decision-making. However, new approaches are needed to be able to fill identified data gaps more quickly and to coordinate researchers and data. Microbial risk assessment is still relatively new and needs continued improvement in quantitative methodology.

- Veterinarians need to be more involved in decisions about antibiotic drug use. There is recognition of the potential financial cost to producers.

- The use of growth promoters needs to be re-evaluated. Do they still work? Why do they still work and how do they work? Previous studies are over 40 years old.

- New approaches are needed for alternatives to antibiotics, not only in product development but also in the re-thinking of animal production and management practices.

- Studies are needed on how to interpret antibiotic resistance transfer on several levels; that is, the transfer of genes, bacteria, and then among larger populations, such as humans and animals.

- There was agreement that all involved individuals need to create collaborative relationships, rather than defensive 'blaming' postures to help solve this complex problem.

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