



**Food and Agriculture
Organization of the United
Nations**



World Health Organization



**World Organization for
Animal Health**

Background Document for the Joint WHO/FAO/OIE Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment, Geneva, Switzerland, 1-5 December 2003

Considering the present state of knowledge of non-human antimicrobial usage and antimicrobial resistance the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (OIE) in 2003 decided to initiate a consultative process to help inform Member States and the Codex Alimentarius Commission on these issues.

Part of the background for this decision lies in previous questions to the Organizations from the Codex Alimentarius Commission: Considering that antimicrobial usage and resistance is a multi-factorial problem and thus requires a multidisciplinary approach, the Executive Committee of the Codex Alimentarius Commission in its 53rd session, recommended that FAO, WHO and OIE should give consideration to convening a multidisciplinary expert consultation. All issues of antimicrobials in agriculture and veterinary use (including aquaculture) should be considered and the role played by antimicrobials as essential human and veterinary medicines should be taken into account. It was agreed that the issues raised by several Committees required a more general and multidisciplinary and multi-agency response.

In preparing for this consultative process the following two-step approach was followed:

- a first workshop dealing with the scientific assessment, considering all uses of antimicrobials in agriculture and veterinary use (including aquaculture) and taking into account the role played by antimicrobials as essential human and veterinary medicines, was held in Geneva, 1-5, December 2003
- a second workshop dedicated to outlining the management issues as well as mitigation strategies, based on the preliminary scientific assessment of antimicrobial use in the food-chain and its consequences for human public health, has been scheduled to be held in Oslo, Norway, 15-18 March 2004

The outcome of the consultative process will be made available for Member States and the Codex Alimentarius Commission to inform future developments in this area.

Prior to the scientific workshop, a group of experts were asked to prepare a background paper. This document is herewith made available on the web-site of the Organizations so as to give additional information related to the first workshop. It does not represent the collective views of the experts in the workshop or any of the Organizations. The final report of the workshop, to which this background document contributed, is available online at: <http://www.who.int/foodsafety/micro/meetings/nov2003/en/>

Background Document for the Joint First FAO/OIE/WHO Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance: Scientific assessment

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Introduction

Throughout history, infectious diseases have been a major threat to human and animal health and a prominent cause of morbidity and mortality. The introduction of antimicrobial agents in the 1930s (sulfonamide) and 1940s (penicillin) completely revolutionized medicine.

Antimicrobial agents are the foundation of modern infectious disease treatment and have played a considerable role in substantially reducing the morbidity and mortality of many infectious diseases.

Antimicrobials have had a more positive impact on human and animal health than any other medical discovery. In the early history of antimicrobial agents, it was perceived that infectious diseases had been conquered and were no longer a major threat to human and animal health. However, it was soon observed that bacteria could develop resistance to antimicrobial agents. Due to development, spread and persistence of antimicrobial resistance, antimicrobial agents are losing their effectiveness.

Antimicrobial resistance is a consequence of antimicrobial use; the use of antimicrobials selects for resistant bacteria in a Darwinian “survival of the fittest” way. Through complex mechanisms, resistance can spread between bacteria. The fact that resistance genes do not respect phylogenetic, ecological or geographical boundaries implies that antimicrobial use and the resulting resistance in one ecological niche may have consequences for the resistance situation in another niche. Thus, antimicrobial resistance in humans and “non-human” environments are interdependent. Consequently, when addressing the problems of antimicrobial resistance one has to take a global and holistic approach that also includes different ecological niches.

A. Objective and purposes of non-human use of antimicrobials

In this report, non-human environments are divided into:

- (Terrestrial) food animals
- Farmed fish (including shellfish)
- Companion animals
- Horticulture

The non-human use in food animals can be divided into therapeutic, prophylactic, metaphylactic and growth promoting use. Table 1 summarizes the types of antimicrobials use in food animals.

Therapy

As in human medicine, a main objective of antimicrobial use in animals, including food animals, pets and farmed fish, is to treat infectious diseases.

Food animals

The treatment is either individual or involves groups of animals. For some animals, such as poultry, mass medication is the only feasible means of treatments. Systemic antimicrobial treatment can be conducted orally, through medicated feed or water, or by injections, usually as an initiation of antimicrobial treatment (followed by systemic or local treatment). Local antimicrobial treatment includes intramammary inoculation for mastitis treatment, intrauterine treatment, and topical skin, ear and eye treatment.

Farmed fish

Antimicrobial treatment is almost always administered to large groups of individuals, although some broodstock may be treated by injection or immersion. Oral treatment is administered through medicated feed that is added to the water where the fish are kept. Usually it involves both diseased fish (therapy) as well as non-diseased fish (prophylaxis). Such mixed use is sometimes called metaphylaxis (see below).

Companion animals

Antimicrobial treatment is usually on an individual basis.

Systemic treatment is conducted orally, by the administration of tablets or mixtures, or by injections. Local antimicrobial treatment includes topical skin, ear and eye treatment.

Horticulture

Antimicrobial use in horticulture is mainly prophylactic. The antimicrobials are administered as spray treatment.

Prophylaxis

Prophylaxis means preventative use of antimicrobials; medication of individuals to avoid development of infections.

Food animals

Prophylactic antimicrobial treatments are typically used during high-risk periods for infectious disease (e.g., after weaning or transport, prior to surgery). The administration may be oral, by injection or intramammary.

Companion animals

Prophylactic antimicrobial treatments are mainly used prior to surgery with high risk of infection. The administration is typically by injection or oral.

Horticulture

The use of antimicrobial in this sector is mainly prophylactic – that is, when disease is expected on the basis of previous experience, predictive systems, or recommendations of local agricultural advisors.

Metaphylaxis

Metaphylaxis is a term used for certain mass-medication procedures, aimed to treat sick animals while medicating others in the group to prevent disease. Typically, metaphylaxis involves administering drugs at therapeutic levels for short periods of time. Metaphylaxis is typically used during outbreaks of infectious disease in fish farming and in poultry. Metaphylaxis is sometimes also used in pigs and cattle.

Growth promotion

Antimicrobial agents are used in food animals to promote growth and enhance feed efficiency. Growth promoters are usually administered in relatively low concentrations, ranging from 2.5 to 125 mg/kg (ppm), depending on the drug and species treated. Such levels that are usually less than therapeutic concentrations are commonly referred to as subtherapeutic doses. In the United States, “subtherapeutic” was defined as uses of antimicrobials in feeds at concentrations <200g per ton for >2 weeks, although use of this term has fallen into disfavor.

How antimicrobials improve growth or feed efficiencies in farm animals is not fully understood. One possibility is that antimicrobial agents dampen the effects of subclinical disease on growth. Another possibility is an antimicrobial effect on anaerobes (which are seldomly susceptibility tested) resulting in curing or preventing certain diseases like necrotizing enteritis or suppressing clostridia and their toxin production. Another possibility is that growth promoters enhance the immune system of recipient animals by affecting hormones, cytokines, and other immune factors.

Antimicrobials at subtherapeutic levels may also modulate the metabolic activity of bacteria in the gut or shift the balance among microbial species, resulting in weight-gain benefits. Although some reports indicate that such uses yield 1%-11% weight-gain improvements, these benefits may not be realized amid other modern production practices. Moreover, such benefits tend to be greater when hygiene is poor.

With improvements in hygiene and other measures in place to control disease (e.g. biosecurity, vaccination, improved management), questions are being raised as to whether intensive animal husbandry practices may eliminate the benefits of growth promoters.

The Danish experience seems to show that in weaning pigs growth promoters have a prophylaxis benefit on *Lawsonia* infections.

In Europe, the legislation for growth promoters use derived from the Swann report (1968), and penicillins, tetracyclines, sulfonamides have not been used for growth promotion. The growth promoters are listed in table 2 and 3. These are mainly active on Gram-positive bacteria and anaerobes, and most of them had no activity on *Enterobacteriaceae*.

An overview of antimicrobial agents

An antimicrobial agent is a chemical compound that in low concentrations can kill or inhibit the growth of bacteria without causing the host (such as a human or an animal) significant damage. Antimicrobial agents can be naturally produced (like penicillin) by a mould or bacterium, semi-synthetic or synthetically made (like the fluoroquinolones). The term antibiotic is often used as a synonym for antimicrobial agent. In this document the latter term is used. Antimicrobial agents have no activity against viruses.

There are hundred of different antimicrobial agents, most of which belong to a few major family groups. The drugs are classified according to their basic chemical structure. Most of the members within a family arise from additions or substitutions of attachments to the drug's core structure.

The major family groups are:

- Beta-lactam subdivided into: penicillins; oxacillins; ampicillins; carbenicillins; ureidopenicillins; cephalosporins of four generations; penems; monobactams; betalactamase inhibitors
- Aminoglycosides
- Tetracyclines
- Macrolides and lincosamides
- Glycopeptides
- Sulfonamides
- Trimethoprim
- Quinolones and fluoroquinolones
- Phenicols
- Streptogramins (virginiamycin, quinopristin+dalfopristin)
- Polypeptides (bacitracin, polymyxin)
- Nitroimidazols
- Steroid antimicrobials (fusidic acid)
- Polyether antimicrobials (ionophores such as narasin, lasalocid and salinomycin)
- Rifamycins

- Nitrofuranes
- Orthosomycines (avilamycin)

Commercially available antimicrobial agents may be referred to by two different names. The generic name is the common family identification provided by chemists, for example “amoxicillin”. The trade name is given to it by the manufacturer and is often used by physicians, veterinarians and pharmacists when prescribing and dispensing the drugs.

Antimicrobial growth promoters

Due to the international scientific attention and documentation in the past decade regarding the public health risks associated with the use of antimicrobial agents as growth promoters in animal husbandry, some countries, including the EU, have banned or are in the process of phasing out such use. This policy is in accordance with the recommendations given by the WHO http://www.who.int/emc/diseases/zoo/who_global_principles/index.htm.

Many of the antimicrobials that have been or still are used as growth promoters are solely used for this purpose (e.g. avoparcin, virginiamycin). Some countries allow that antimicrobials that are used therapeutically also can be used as growth promoters in sub-therapeutic doses. In the US, antimicrobials such as penicillin, erythromycin, tylosin and tetracycline are approved both for growth promotion as well as therapeutic use.

The follow-up in European countries of resistance in Gram-positive bacteria and their relationship with the ban of growth promoters is important.

Coccidiostats

Coccidiosis, caused by single-celled parasites called coccidian, is an important disease in animal husbandry, particularly in poultry production, that is faecally-orally transmitted. Modern slaughter chicken and slaughter turkey production depends prophylaxis against coccidiosis to be cost-effective. However, vaccines against coccidiosis are increasingly available.

The coccidiostats include quinoxalines, efrotomycine, avilamycine, bambermycin and the ionophores monensin (turkeys), narasin (broiler), lasalocid and salinomycin. In addition to their anticoccidial activity, these drugs possess an antibacterial effect (against some Gram positive bacteria and anaerobes). Thus, coccidiostats represent a sub-group within the antimicrobial agents. Coccidiostats are not only used for their anticoccidial effect, but also as growth promoters due to their effect in improving feed conversion efficiency.

Data indicate that exposure to narasin causes the development of decreased susceptibility to narasin in clostridia (ref) and enterococci (NORM/NORM-VET 2002, SVARM 2002). Little is known regarding resistance mechanisms for ionophores, and whether ionophore resistance might be linked to resistance to other antimicrobials.

Antimicrobials as disinfectants in consumer products

Alcohols, chlorine and peroxides and quarternary ammonium compounds have been used for many decades in health-care and cleaning products. Within the past two decades, the residue-producing antibacterials, such as triclosan, once used almost exclusively in health care institutions, have been added to increasing numbers of household products, particularly soaps, and cleaning agents. <http://www.tufts.edu/med/apua/>

A recent survey reported that 76% of liquid soaps from 10 states in the US contained triclosan and approximately 30% of bar soaps contained triclocarban. Many cleaning compounds contain quaternary ammonium compounds. More recently, triclosan has been bonded into the surface of many different products with which humans come into contact, such as plastic kitchen tools, cutting boards, highchairs, toys, bedding and other fabrics. <http://www.tufts.edu/med/apua/>

Recent studies have shown that some bacteria can combat triclosan and other biocides with export systems that could also pump out antimicrobial agents. It was demonstrated that these triclosan-resistant mutants were also resistant at low level to several antimicrobial agents, specifically chloramphenicol, ampicillin, tetracycline and ciprofloxacin. This field of antimicrobial use needs further attention, and more research in this area is needed. However, antimicrobials as disinfectants in consumer products will not be further addressed in this consultation. <http://www.tufts.edu/med/apua/>

Heavy metals

The addition of copper and zinc in animal feeding stuffs may also select for efflux pump in bacteria conferring low level of resistance to unrelated antimicrobial agents. Mercury and arsenic may raise as environmental pollutants similar problems.

B. Antimicrobial resistance – general aspects

Genetic mechanisms

Antimicrobial resistance is a natural phenomenon developed by bacteria as a mean to escape the antimicrobial effect and to survive its contact.

Emergence and dissemination of antimicrobial resistance is a consequence of antimicrobial use; the use of antimicrobials selects for resistant bacteria, allowing antimicrobial resistant bacteria to survive and multiply.

Antimicrobial resistance is either an intrinsic - naturally occurring - trait or it is acquired. The former type refers to inherent features of the bacterial cell that prevent antimicrobial action, and these properties are typically species characteristics. An example is the resistance of *E. coli* to penicillin G. The term antimicrobial resistance usually refers to acquired resistance, which occurs when resistant strains merge from previously susceptible bacterial populations, usually after selective pressure exerted by antimicrobial agents.

Acquired resistance can arise in many ways. Traditionally, acquired resistance has been divided into two major types; mutational and transferable resistance. However, with increasing knowledge about resistance mechanisms, the distinction between these two types of resistance has become less obvious. A mutation is a random genetic change in existing DNA, and the resultant alteration may render a bacterium resistant to a specific class of antimicrobials. Such a resistance is heritable, spreading vertically, but is generally not transferable. Mutational resistance can develop for all kinds of antimicrobials, but is especially noted for quinolones.

Transferable resistance has been described for the majority of antimicrobials, and for most agents such resistance is usually the most prominent type of resistance among clinical bacterial isolates. Bacteria can acquire antimicrobial resistance genes from other bacteria in several ways. By undergoing a simple mating process called *conjugation*, bacteria can transfer genetic material, including genes encoding resistance to antimicrobial agents, from one bacterium to another.

Resistance genes can also be passed between bacteria by viruses called bacteriophages. Such a mechanism of gene transfer is called *transduction*. Finally, bacteria can acquire resistance genes through the uptake of naked, “free” DNA from the environment, so called *transformation*. Horizontal spread of antimicrobial resistance is phenomenon frequently occurring among closely related bacteria. However, transfer also occurs between distantly related bacteria. Thus, resistance genes do not respect phylogenetic borders. Furthermore, resistance genes can spread between bacteria belonging to different ecological niches, for example hospitals and marine sediments. Last but not the least, resistance genes can be carried by bacteria and spread across national boundaries by movement of people, animals, feed, and food.

Resistance genes that spread between bacteria reside on genetic elements such as plasmids, transposons or gene cassettes that are mobile themselves or can be mobilized. Since various types of resistance genes can be located on the same genetic elements, horizontal spread of resistance can result in the recipient bacteria becoming resistant to several antimicrobials at the same type. Furthermore, bacteria

can collect various resistance traits over time. Contemporaneous resistance to several different antimicrobials is usually referred to as *multi-resistance*.

Cross-resistance denotes the phenomenon when resistance to one antimicrobial confers resistance to another antimicrobial, usually in the same class. For example, cross-resistance typically occurs between various fluoroquinolones and can also occur between different cephalosporins, penicillins, aminoglycosides and macrolides and between macrolides and lincosamides.

Co-selection is a term used when selection for one genetic trait at the same leads to the selection for another genetic trait due to a genetic linkage between the two traits. For instance, the selection for copper resistance exerted by the use of copper as a feed additive in poultry feed may co-select for vancomycin and erythromycin resistance.

Biochemical mechanisms

Biochemical mechanisms of antimicrobial resistance can be divided into three main categories that can be further subdivided (table 4). Several mechanisms of resistance exist for most antimicrobials. In fact, different mechanisms can augment the resistance to a particular drug in a single bacterial isolate.

- Decreased accumulation of the antimicrobial agent
 - Decreased drug entry (decreased permeability) – e.g., mutations in porin genes resulting in low-level resistance to several antimicrobial
 - Increased drug efflux – e.g., active efflux of tetracycline
- Drug inactivation (enzymatic modification) – e.g., beta-lactamases
- Modification of the antimicrobial target site
 - Alteration of the drug target (reduced affinity) – e.g., mutation in DNA gyrase resulting in quinolones resistance and changes in penicillin-binding proteins resulting in penicillin resistance
 - Substitutions of targets insusceptible to antimicrobial agent – e.g., production of new target enzyme resulting in resistance to trimethoprim of sulfonamides
 - Overproduction of drug target – e.g., overproduction of normal target enzyme for trimethoprim so that higher concentration is needed
 - Absence of drug target – e.g., intrinsic resistance in some species to certain antimicrobials

The only resistance mechanisms leading to removal of an antimicrobial agent from the environment, is the action of inactivating enzymes such as beta-lactamases. Resistance to drugs like macrolides, trimethoprim, sulfonamides, quinolones and tetracyclines usually do not result in the destruction of the drug. Thus, these drugs remain intact in the environment until physically destroyed and meanwhile they can kill off susceptible bacteria in their surroundings and select for resistant strains.

Several national and international expert panels have previously addressed the human health impacts of antimicrobial resistance from non-human uses of antimicrobial agents (refs below). This report is a further review and analysis of the scientific information that bears on this issue, and focuses on the

scientific information relevant to future considerations of resistance risk management. It builds on previous expert reports and incorporates more recent information, including unpublished data solicited by OIE/WHO/FAO for this consultation

Origin of resistance genes

Origin of resistance genes is debated: it may originate from naturally resistant bacteria or from microorganisms that are producers of antibiotics, by a mechanism of genes picking-up and recombination.

Where does the transfer of genes occur?

The food animal gut and the human gut have been considered as the important location for genes exchanges. However, environmental bacterial niches, soil, river, pets, wild rodents, and fish, are also location where genes transfer occurs. Few studies have compared, the residual activity of antimicrobial agents and its duration, in environmental conditions.

The complexity of the pathways for resistance origin and spread will be discussed in another chapter.

Pattern of usage

The pattern of usage of antimicrobial agents is extremely different between countries, regions and farms. Prophylaxis and metaphylaxis are administered with different rationales. Duration of administration of subtherapeutic doses and time in the animal life are also diverse. To a lesser extent, therapeutic administration of antimicrobial agents can be variable.

It is essential to establish and to promote any antimicrobial usage on scientific background: pharmacokinetic, and pharmacodynamic, with clinical trials properly conducted should establish the base for antimicrobial use, in their different indications.

Studies exploring the characteristics of the pattern of use in a country, will optimize the impact and their implementation of recommendations aiming to the “Prudent Use of antimicrobials”.

Since resistance is spreading between humans, animals, environment, there is need to explore the distribution of resistant bacteria, not only in animals and humans (pathogens and commensals) but also in environmental niches, for a better and efficient use of antimicrobial agents in animals. Furthermore, more studies on prevalence of resistance in animal pathogens and on their therapeutic implication are needed.

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Table 1: Types of antimicrobial use in animals

Type of antimicrobial use	Purpose	Route or vehicle of administration	Administration to individuals or groups*	Administration to diseased animals
Therapeutic	Therapy	Injection, feed, water	Individual or group	Diseased individuals; in groups, may include some animals that are not diseased or are subclinical
“Metaphylactic”	Disease Prophylaxis, Therapy	Injection (feedlot calves), feed, water	Group	Some
Prophylactic	Disease prevention	Feed	Group	Not evident, although some animals may be subclinical
“Subtherapeutic”	Growth promotion	Feed	Group	No
	Disease prophylaxis	Feed	Group	No

*Food animals are usually grouped by pen, flock, pond, barn, or other aggregate.

Modified from SA McEwen and PJ Fedorka-Cray. Antimicrobial use and resistance in animals. Clinical Infectious Diseases 2002; 34: S93-S106.

Table 2: Examples of antimicrobials approved for use in the United States in food animals

Purpose	Cattle	Swine	Poultry	Fish
Treatment of various infections	Amoxicillin Cephapirin Erythromycin Fluoroquinolone Gentamicin Novobiocin Penicillin Sulfonamides Tilmicosin Tylosin	Amoxicillin Ampicillin Chlortetracycline Gentamicin Lincomycin Sulfamethazine Tiamulin Tylosin	Erythromycin Fluoroquinolone Gentamicin Neomycin Penicillin Spectinomycin Tetracyclines Tylosin Virginiamycin	Ormetoprim Sulfonamide Oxytetracycline
Growth promotion	Bacitracin Chlortetracycline Lasalocid Monensin Oxytetracycline	Asanilic acid Bacitracin Bambermycin Chlortetracycline Erythromycin Penicillin Tiamuline Tylosin Virginiamycin	Bambermycin Bacitracin Chlotetracycline Penicillin Tylosin Virginiamycin	

Table 3. Growth promoters in Europe

Antibiotics	Banned since	Class	Selected antimicrobial agents in humans
Bambermycin		Glycolipid	
Bacitracin	1999	Cyclic peptide	+ (Topically)
Monensin		Ionophore	
Salinomycin		Ionophore	
Virginiamycin	1999	Streptogramms	Quinupristine/dalfopristine
Tylosin	1999	Macrolide	Erythromycin
Spiramycin	1999	Macrolide	Erythromycin
Avilamycin		Orthosomycin	
Avoparcin	1997	Glycopeptide	Vancomycin-teichoplanin
Ardacin	1997	Glycopeptide	Vancomycin-teichoplanin
Efrotomycin		Elfamycin	
Olaquinox	1999	Quinoxaline	
Carbadox	1999	Quinoxaline	

Table 4: Resistance mechanisms, the affected antimicrobial agents and the resulting level of resistance

Mechanism	Affected antibiotics	Level of resistance
Efflux	Tetracyclines	Low
	Macrolides	
	Quinolones	
	Others in different systems	
Penetration	B-lactams	Low
	Chloramphenicol	
	Trimethoprim	
	Tetracyclines	
Target Alteration	B-lactams	Variable
	Aminoglycosides	
	Macrolides	
	Quinolones	
	Rifampicin	
	Glycopeptides	
By-pass	Sulphonamides	High
	Trimethoprim	
Enzyme detoxification	B-lactams	High
	Aminoglycosides	
	Macrolides	
	Chloramphenicol	
	Lincosamide	

Chapter 1: Surveillance of usage

A. Recommendations from previous FAO, OIE and/or WHO consultations/meetings

In October 1997, WHO convened an expert meeting on “The Medical Impact of the Use of Antimicrobials in Food Animals” in Berlin (<http://www.who.int/emc/diseases/zoo/oct97.pdf>). The objective was to achieve, if possible, an international consensus on priority medical problems arising from the use of antimicrobials in livestock production. The meeting concluded that the use of antimicrobials in food animals is a public health issue on which prudent use guidelines should be implemented, and that monitoring of both antimicrobial resistance as well as antimicrobial usage is warranted. Among others it was recommended that:

- the use of any antimicrobial growth promoters should be terminated if they are used as human therapeutics, or known to select for cross-resistance to antimicrobials used in human medicine
- a systematic approach aiming at replacing growth-promoting antimicrobials with safer non-antimicrobial alternatives should be established
- no antimicrobial should be administered to a food animal unless it has been evaluated and authorised by competent national authorities
- national authorities should maintain records of export/import figures of bulk chemicals with potential antimicrobial use, as such information is vital for quantitative assessments of the medical risks related to the use of antimicrobials in livestock production

Subsequently, in June 2000, WHO convened with participation of FAO and OIE a consultation in Geneva, and a consensus agreement was reached on principles aimed at minimizing the potential negative public health impact of the use of antimicrobials in food producing animals while recognizing the ongoing need for antimicrobial treatment of diseased animals. These principles were subsequently published as “WHO global principles for the containment of antimicrobial resistance in animals intended for food” (http://www.who.int/emc/diseases/zoo/who_global_principles/index.htm). Forty principles were agreed upon. These covered such areas as:

- the responsibilities of regulatory and other relevant authorities
- quality and manufacturing
- distribution/sales and marketing
- antimicrobial growth promoters
- surveillance of antimicrobial resistance and antimicrobial usage
- prudent use of antimicrobials
- prophylactic use of antimicrobials
- education and training
- research.

Two specific sections specifically addressed the issue of monitoring the usage of antimicrobials:

1. The surveillance of antimicrobial resistance and antimicrobial usage:

“Data generated from the surveillance of antimicrobial resistance and antimicrobial usage should play a key role in the development of national policies for the containment of antimicrobial resistance. These data are all essential in the pre- and post-licensing process and in the development and treatment guidelines for veterinary use.”

2. The surveillance of antimicrobial usage:

“Relevant authorities should establish systems to determine the amounts of antimicrobials given to food animals.”

“Information on the amounts of antimicrobials given to food animals should be made publicly available at regular intervals, be compared to data from surveillance programmes on antimicrobial resistance, and be structured to permit further epidemiological analysis.”

In the WHO Global Principles document it was stressed that “the real challenge will be to translate the Global Principles into national rules and regulations, codes of practices and standard operating procedures. This will only occur if we succeed in engaging in an open, transparent and collaborative effort at national as well as international level, bringing together all stakeholders in the complex process of reducing health risks from the misuse and overuse of antimicrobials in animals intended for food.” As a follow-up, WHO convened in September 2001 a consultation on the monitoring of antimicrobial usage in food animals for the protection of human health in Oslo, Norway. This consultation built on earlier WHO consultations and recommendations. It was acknowledged that data generated from the monitoring of antimicrobial usage and antimicrobial resistance play a key role in the:

- development of national and international (for example WHO, the Food and Agricultural Organization of the United Nations – FAO, Codex Alimentarius and the *Organization Internationale des Epizooties* - OIE) policies for the containment of antimicrobial resistance
- comparison of the usage of antimicrobials at different levels (local, regional, national, international)
- informing and in the education of stakeholders
- correlation with data from antimicrobial resistance monitoring in humans, animals, and food
- application of risk analysis processes pertaining to the issue of antimicrobial resistance
- evaluation of the impact of the implementation of the prudent use of antimicrobials and of other interventions

The report from the Oslo consultation provides a summary of the presentations that were made there. (http://www.who.int/emcdocuments/antimicrobial_resistance/whocdscsreph200211.html)

This summary gives a useful overview of the situation regarding monitoring of antimicrobial usage in various parts of the world. The consultation resulted in a set of recommendations on the monitoring of antimicrobial usage in food animals for the protection of human health:

1. Countries should establish a national monitoring programme of the usage of antimicrobial agents in food animals

2. Countries should have a regulatory approval and control system for antimicrobial agents and products containing antimicrobial agents
3. Countries should collect data on the total amounts of each antimicrobial agent and report these data in kilograms of active ingredient on an annual basis
4. WHO in collaboration with other relevant international organizations should recommend a system to identify and classify antimicrobial agents and quantify their use in order to make data comparable.
5. Countries should link antimicrobial usage data with antimicrobial resistance data.

In November 2002, WHO convened an independent, multidisciplinary, international expert panel to review the potential consequences to human health, animal health and welfare, environmental impact, animal production, and national economy resulting from Denmark's program for termination of the use of antimicrobial growth promoters in food animal production, particular swine and broiler chicken. This panel was held in conjunction with the International Invitational Symposium: Beyond antimicrobial growth promoters in food animal production (6-7 November 2002, Foulum, Denmark). <http://www.who.int/salmsurv/links/gssamrgrowthreportstory/en>

Based on information obtained by comprehensive monitoring of antimicrobial use, the panel concluded that Denmark's programme to discontinue the use of antimicrobial growth promoters has been very beneficial in reducing the total quantity of antimicrobials administered to food animals.

In May 2003, the OIE International Committee approved four documents that constitute the "OIE guideline on antimicrobial resistance". Document number three deals with antimicrobial usage: Monitoring the quantities of antimicrobials used in animal husbandry (Vose et al 2001).

B. Surveillance of antimicrobial usage

Purposes

The FAO, OIE and WHO have recommended that each country should implement a surveillance programme on antimicrobial usage in animal husbandry. The system, which should be under the responsibility of a competent national authority, should be clear and transparent as this will facilitate data comparison within and among countries as well as interpretation of trends. The purposes of monitoring antimicrobial usage are manifold and include:

- Documentation of the situation as regard quantities of antimicrobial used as well as usage patterns (more or less detailed depending on how sophisticated the monitoring system is)
- Identification of trends in the use of different antimicrobials (overall or by animal species)
- Explanation of patterns and trends as regard antimicrobial resistance
- Explanation of patterns and trends as regard antimicrobial residues
- Basis for risk assessment as regard antimicrobial resistance
- Basis for risk assessment as regard antimicrobial residues
- Basis for interventions

- Evaluation of effectiveness of measures implemented (e.g., campaigns on prudent use, termination of antimicrobial growth promoters drugs, mitigation strategies)
- Basis for focused and targeted research

Methodology – general aspects

Data sources for surveillance antimicrobial usage

Different data can be used as input in a surveillance system on antimicrobial usage. The type of input data will again influence the degree of detail in the output data. Sources of data, to which degree data are obtainable, and the methods on how to collect or obtain usage data will vary from country to country because different countries have different distribution and registration systems. Access to data may require legislative support. Furthermore, economic compensation or support may be necessary.

Overall national usage data

A simple and cost effective surveillance system on antimicrobial usage can be achieved based upon data on overall usage for the various antimicrobial formulations. The following sources for obtaining data for the estimation of overall usage may be utilized:

- Import and export registration
- Pharmaceutical industry
- Wholesalers
- Feed mills
- Pharmacies

Import data or overall national sales statistics does not give information as regard animal species, farm, geographical area, or clinical indications. However, a large proportion of the veterinary antimicrobial drugs may be species specific, making it possible to make rough estimate on usage in the different species based on overall statistics (Grave K et al, 1996; Grave K et al, 1999; Grave K et al., 2003). Furthermore, overall national sales data may be split into geographical regions, e.g., communities and counties, if the national drug distribution system allows for this. Moreover, overall usage data represent several opportunities as regard pharmacoepidemiological studies. Last but not least, overall usage data are important and necessary for the validation of other data sources.

Usage per species, at herd level, etc.

More sophisticated surveillance systems might make use of stratified data that gives information on usage for various animal species, usage at herd level, usage in relation to indications and in various regions. Data sources for such a stratified surveillance system may include:

- Pharmacies (prescription based data)
- Veterinarians
- Feed mills
- Farmers and food animal producers

These sources may be appropriate when pharmaceutical industry or wholesalers cannot be used for the routine collection of antimicrobial usage data or when more accurate and locally specific information is required. A prescription based surveillance system will provide information about animal species and can also provide details about geographical area, farm, herd and clinical indications. However, the implementation of such an advanced system seems most feasible in countries where the veterinary drugs are dispensed by pharmacies (Steger et al., 2003).

Collection, storage and processing of stratified data have to be carefully designed and well managed, for example in sentinel studies. However, such systems should have the advantage of producing accurate and targeted information. Periodic or targeted collection of this type of data may be sufficient. Factors such as seasonality and disease conditions, species affected, agricultural systems (e.g., extensive range conditions and feedlots), may be important factors when designing such studies.

Identification and classification of antimicrobials

Countries should have a regulatory approval and control system for all antimicrobial agents and products containing antimicrobial agents used in animals. Such a system could include, but not be limited, to listing of all available antimicrobial agents in the country and an approval mechanism.

Ideally, all classes and substances of antimicrobial should be included in a surveillance programme. If this is not possible, decisions need to be made on what classes of antimicrobials should be considered. Standardized national and international terminology and methodology of reporting is essential so that it is clear which antimicrobials are monitored and used. A system is required to identify and classify antimicrobials similar to the ATC (Anatomical Therapeutic Chemical) classification system, which is used for human antimicrobials. ATCvet is the parallel system for veterinary antimicrobials. It is recommended that this classification system or a corresponding system should be used in the identification of specific antimicrobials.

Units of measurement

Kilogram active ingredient

Data should be collected to minimum express the annual weight in kilograms on the active ingredient of the antimicrobial(s). If a country has the infrastructure for capturing basic animal antimicrobial use data for a specific antimicrobial, then additional information can be considered to cascade from this in a series of subdivisions or levels of detail. Such a cascade of levels should include the following:

- i) The absolute amount, in kilograms of active ingredient, used for a specific antimicrobial chemical entity or minimum for class (-es) of antimicrobials per year
- ii) Therapeutic and growth promotion usage expressed as kilograms of the different antimicrobial ingredients.
- iii) Subdivision of the antimicrobial usage data into therapeutic and growth promotion use by species.
- iv) Subdivision of the antimicrobial usage data into routes of administration, specifically in-feed, in-water, injectable, oral, intramammary, and intra-uterine
- v) Subdivision by indication, animal species, age group

- v) Further subdivision of these figures by season and region may be useful
- vi) Further breakdown of data for analysis of antimicrobial use at regional, local, herd and individual veterinarian level may be possible using veterinary practice computer management software as part of specific targeted surveys or audits. Analysis of this information within the local or regional context could be useful for individual practitioners and practices where specific antimicrobial resistance has been identified and feedback is required.

Prescribed daily dose (PDD), defined daily dose (DDD) and course dose

Further research and development is needed to develop units of measurement that most accurately describe the antimicrobial selective pressure, in order to facilitate epidemiological analysis of usage data relative to antimicrobial resistance data and to support the comparison between different animal species, over time and with human usage. Data collected on-farm or from veterinarians may be expressed as prescribed daily dose (PDD) per weight of animal (Chauvin C et al 2002; Arnold S et al 2003) or per animal at risk and may be expressed as prevalence or incidence estimates. In human medicine, defined daily dose (DDD) is used to interpret overall sales figures of drugs because this unit allows for the comparison of usage of antimicrobial drug of varying potency (WHO, 2002). The DDD concept may be used in veterinary medicine to express prescribing patterns if usage data or estimates of usage per species are available (Grave K et al 1999; Anon., 2003, Grave K et al 2003). Furthermore, where data on animals at risk are available, incidence of use or treatment frequency may be estimated by transferring the DDDs into course doses (Grave K et al 2003).

Species, production systems and census data

Countries should keep a register of all animal use antimicrobials for specific food animal species (cattle, sheep, goats, pigs, poultry, horses and fish) and for specific diseases. This will help to identify possible non-authorized usage. For use in risk assessments and to facilitate data comparison within and between countries as well as interpretation of trends, data on animal population and production should be provided, for example numbers of animals in the various categories slaughtered per year or animal census data.

Surveillance usage in food animals, including aquaculture

During the Oslo consultation 2001, it became obvious that the level of control with antimicrobial usage in food animals is highly variable between different countries and that the feasibility of implementing surveillance schemes for antimicrobial usage differs greatly between countries. <http://www.who.int/emc/diseases/zoo/antimicrobial.html>

Speakers from developing countries frequently reported a lack of control with antimicrobial use in food animals. In India, there is no legislation regarding antimicrobial usage or residues in food. A recent survey of Indian water buffalo milk revealed high levels of oxytetracycline residues, indicating that antimicrobial use is widespread and under little control. A similar situation was reported from Indonesia, as well as in other countries in the same area. Thailand has a large production of swine and poultry, where many antimicrobials are applied for growth promotion purposes. The Thai aquaculture industry, with production of shrimp in particular, routinely uses large quantities of antimicrobials.

In African countries such as the United Republic of Tanzania and Uganda, veterinary antimicrobials are easily accessible and under low levels of control from government authorities. The fact that expired antimicrobials have been given new labels and subsequently exported to developing countries is another issue of concern. An extensive study performed in Kenya between 1995 and 1999 estimated the use of antimicrobials to food animals using data from both local manufacturers and official import records. This work stands to prove that reasonably accurate estimates of antimicrobial usage can be obtained without high costs, as long as there is a transparent regulation of the pharmaceutical trade present. Overall, a strong need for control measures and prudent use guidelines was emphasized by several speakers from developing countries.

The knowledge about the amount of antimicrobials used in agriculture in the US is limited, making it difficult to determine which drugs are used in what quantities and for what purposes. The Union of Concerned Scientists has published a report that estimates the non-therapeutic usage of antimicrobials in livestock in the US exceeds the human use by far (8 times) (Mellon M et al 2001). The report estimated that 3 million pounds of antimicrobials are administered to humans annually, whereas 27.5 million pounds of antimicrobials are used for “non-therapeutic” purposes (growth promotion and disease prophylaxis) and another 2 million pounds are used for therapeutic purposes in animals. In addition, it was estimated that 1.5 million pounds are used in soaps, topical creams and disinfectants. However, all these figures were based on extrapolations and indirect methods using publicly available information like herd sizes, indications for antimicrobial use and dosages, and not actual sales figures. The Animal Health Institute in US has also estimated the antimicrobial usage in livestock and suggests that in 1998, 17.8 million pounds of antimicrobials were used in animal production; 14.7 million pounds (83%) for prevention and treatment of disease, and 3.1 million pounds (17%) for growth promotion (Animal Health Institute 2000). There are several obstacles to obtaining correct estimates of the antimicrobial usage in US agriculture. Many drugs that are used in food-producing animals require no prescription and are sold straight from manufacturers to distributors without going through a pharmacy. Also, when the sponsors of approved animal drugs in the United States submit their annual reports on the sales of each drug they are not required to specify whether the substance is meant for domestic use or export or what the actual conditions for use are. The US Food and Drug Administration is proposing changes in this recording system to enable a more accurate estimate of the antimicrobial usage in food animals.

In Australia all antimicrobials are imported. The government requires details from importers on the intended end-use of all these imported antimicrobials. Hence there is data available on the quantities of different antimicrobials used in animals and in people within that country.

Denmark established in 2001 VetStat, the most advanced surveillance programme on antimicrobial usage in animals worldwide. VetStat is a programme for prescription-based surveillance of usage of veterinary medicines. This end-point surveillance of antimicrobial usage allow for obtaining details about the usage, such as target species, age class of animals treated and main indication for use. In addition to prescription-medicines, VetStat also monitors usage of feed additives, coccidiostats and antimicrobial growth promoters. In Denmark, prescription medicines are available only through pharmacies or – for a few medicines approved for use in medicated feed – through licensed feed mills, facilitating data collection. Data from VetStat are published together with antimicrobial resistance data in the annual DANMAP report http://www.vetinst.dk/file/2/DANMAP_2002endelig.pdf. This comprehensive system has placed Denmark at the pinnacle of antimicrobial usage surveillance in food animals.

Other countries in Europe have also implemented surveillance programmes for usage of antimicrobial drugs in food animals, but these are much simpler as compared to VetStat and are generally based upon data from wholesalers (Anon. 2003a, Anon. 2003b, NORM/NORM-VET report 2002, SVARM 2002, VMD). As regard farmed fish, reliable data show that the total antimicrobial use in Norwegian aquaculture declined by 98% during the period 1987-2002, while the production of farmed fish increased massively. This significant decrease of antimicrobial use in aquaculture is attributed to the introduction of effective vaccines and improved health management. Norway established already in 1989 a prescription based surveillance of antimicrobial usage. This system allows for obtaining details about all antimicrobial usage in Norwegian aquaculture, including indications and locations (Grave K et al 1996).

On September 29, 2003, the European Union adopted new EU legislation designed to improve protective measures against zoonoses. www.health.fgov.be/WHI3/krant/krantarch2003/kranttekstsept3/030930m12eu.htm. The legislation, which will enter into force on its day of publication in the Official Journal of the European Union, lay down requirements as regard surveillance and control of various zoonoses. Furthermore, it is stated that antimicrobial resistance should be monitored, and that this surveillance in addition to zoonotic agents might include other relevant agents, including indicator bacteria. This new legislation does not deal with usage surveillance *per se*. However, for countries to be able to interpret resistance data properly, knowledge about usage is critical. Thus surveillance of resistance will necessitate some degree of usage surveillance.

The Oslo consultation concluded that because various countries have such different levels of control with the antimicrobial usage for animals intended for food, some system of standardization is needed to enable comparisons of usage between countries and regions. Both units of measurement and the categories of antimicrobials need to be defined and standardized.

Surveillance usage in companion animals

The population structure and the pattern of antimicrobial usage in companion animals resemble that of humans, more so than that of food animals. Antimicrobial agents are widely used in small animal practice as therapeutic agents. For instance, long and repeated antimicrobial treatment is often required for treatment of chronic skin infections and otitis externa, both of which are common diagnoses in dogs. Quite often antimicrobial preparations licensed for humans are applied in companion animals, including more sophisticated antimicrobials that are important in human medicine such as cephalosporins and fluoroquinolones. However, few countries have good data as regard the antimicrobial usage in companion animals as this will require that a prescription based surveillance programme is in place (Odensvik et al., 2001). Only for antimicrobial formularies specifically intended for companion animals it will be possible to obtain usage data through sales data from wholesalers. The usage of human preparations is complicating the issue of surveillance usage in companion animals as such use will affect the statistics on human drug consumption and not usage in animal.

Antimicrobial usage in companion animals might not seem relevant in regard to public health, and food safety in particular, at first sight. However, the close proximity of companion animals and owners provides opportunities for exchange of bacteria and resistance determinants amongst these populations. Accordingly, intensive use of antimicrobials in companion animals could present a hazard to the human

population by promoting the development of resistant bacteria, which subsequently can be transmitted to their owners either directly by physical contact (petting, licking, physical injuries, etc.) or indirectly through the domestic environment (contamination of food, furnishings, etc.). For example, food preparation in the family kitchen represents a potential for spread of bacteria from the companion animals to its owner. Recent research has shown that transfer of multiple antimicrobial resistant *Staphylococcus intermedius* and quinolone resistant *Campylobacter jejuni* can occur between humans and dogs living in the same household (Guardabassi L et al. 2003, Damborg P et al. 2003). Thus, antimicrobial usage in companion animals may ultimately have a public health effect. It has also been shown that dogs, independent of the source of infection, can act as important reservoirs of methicillin resistant *Staphylococcus aureus* (MRSA) within family households. Recurrent MRSA infection has been recently reported in a human patient with diabetes and his wife's wound. Culture from the nasal cavity of the family dog showed the presence of the same MRSA strain, probably spread from the owners to the dog, and recurrence of the infection in the couple was stopped after eradication of the organism in the dog (Manian FA 2003). The role of companion animals as reservoirs of resistant bacteria should be more carefully considered and studied in view of the usage of antimicrobials in small animal practice and the close proximity of companion animals and owners.

Surveillance usage in horticulture

Bacterial diseases of plants are less prevalent than diseases caused by fungi and viruses. Antimicrobials for prophylactic treatment of bacterial diseases of plants are limited in availability, use, and efficacy, and therapeutic use is largely ineffective. In the US, the Environmental Protection Agency (EPA) has regulatory responsibility for antimicrobial use in plants. Only two antimicrobials, streptomycin and oxytetracycline, are currently registered by the EPA for use in horticulture. Both are used primarily as prophylactic treatments – that is, when disease is expected on the basis of previous experience, predictive systems, or recommendations of local agricultural advisors. Streptomycin is registered for use on 12 fruit, vegetable, and ornamental fruit crops, and oxytetracycline is registered for use on four fruit crops. Some data on minor uses for other crops and seed treatment are not available (Vidaver AK 2002). Most applications are by spray treatments in orchards to prevent fire blight, a major plant disease caused by *Erwinia amylovora*.

Approximately 53,000 hectares (131,000 acres) are sprayed annually with antimicrobials in the US. Use in fruit orchards accounts for less than 50,000 pounds annually, whereas the total use in vegetable production accounts for less than 5000 pounds (Mellon et al. 2001). In 1999, according to the US Department of Agriculture, 30% of the pear acreage received a total of 6000 pounds of streptomycin and 40% of the acreage received a total of 12,000 pounds of oxytetracycline. Apples received >15,000 pounds of streptomycin on 20% of the acreage, or 3000 pounds of oxytetracycline on 5% of the acreage. In 1997, 39,800 pounds of streptomycin and 26,800 pounds of oxytetracycline were used, mostly on pears and apples. The total use of antimicrobials on fruit crops in the US increased by 2.1% from 1991 to 1999 (Mellon et al. 2001). Streptomycin use decreased during the 1990s, whereas oxytetracycline use increased, except in 1999. One reason for the increased use of oxytetracycline is the increasing prevalence of streptomycin resistance in the target bacterium, *E. amylovora* (Vidaver AK 2002).

Resistance to streptomycin has become widespread among bacterial phytopathogens, but no resistance among these bacteria has yet been reported for oxytetracycline. However, surveillance of antimicrobial resistance in plant pathogens is not routinely done (Vidaver AK 2002). A transposon

carrying a gene for streptomycin resistance apparently evolved in *E. amylovora* in response to the spraying of apple orchards with streptomycin. This transposon has later been found in *E. coli* in pigs where a gene for trimethoprim also has been inserted. The appearance of a new resistance gene under heavy selection pressure from trimethoprim used in pig rearing and borne on a transposon earlier found in the very different context of a plant pathogen, illustrates the powerful ability of micro-organisms to effect a horizontal flow of genetic material mediating resistance http://biosafety.ihe.be/ARGMO/Documents/Opinion_SCAN_EC_out64_en.pdf. Residue studies showed that fruit had no detectable streptomycin residue at the time of harvest, but streptomycin activity was still detectable in leaves (Vidaver AK 2002).

In several Central and South American countries gentamicin is used in food crops. However, no data are available on gentamicin use in agriculture in these countries or on the occurrence of antimicrobial resistance of bacteria on fruits and vegetables from this region (Vidaver AK 2002). The US EPA does not allow importation of fruits and vegetables treated with gentamicin because of the importance of gentamicin in human medicine.

In China, the use of a wide range of antimicrobials in the production of fruit, rice, rubber, tobacco, cabbage, cucumber and other agricultural products have been reported (WHO, 1997).

C. Data gaps and areas for improvement

It is evident from the above that in most countries the data available on non-human antimicrobial usage in general is very limited and usually encumbered by significant uncertainties. This is very unfortunate as usage data are important in order to better understand the epidemiology of antimicrobial resistance and to use as a basis for risk assessment which again are used to base risk management actions on.

Further research and development is needed into methods to analyze usage data and to facilitate the comparison between different animal species, geographical areas, per time period and with human use. To interpret usage data retrieved per species, a DDD unit of measurement for the most important food animal species should be agreed on.

D. Summary/conclusions/recommendations

There is a need for countries to establish surveillance systems that allows for obtaining reliable data on non-human antimicrobial usage. As a minimum, national data on overall usage for the various formulations should be collected. Countries should link antimicrobial usage data with antimicrobial resistance data, preferably within the same animal species.

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Chapter 2: Surveillance of Antimicrobial Resistance in Food and Food Animals.

A. Recommendations from previous FAO, OIE and WHO Consultations and Meetings

The WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (“Global Principles”) provide a framework of recommendations to reduce the overuse and misuse of antimicrobials in food animals for the protection of human health. The overall objective of the Global Principles is “*To minimize the negative public health impact of the use of antimicrobial agents in food-producing animals whilst at the same time providing for their safe and effective use in veterinary medicine*”. The Global Principles were developed with the participation of the Food and Agriculture Organization of the United Nations and the Office International des Epizooties, as part of a comprehensive WHO Global Strategy for the Containment of Antimicrobial Resistance and are available at http://www.who.int/emc/diseases/zoo/who_global_principles/index.htm. The foundation for the WHO Global Principles was laid during several previous consultations, including the WHO Consultation on the Medical Impact of the Use of Antimicrobials in Food Animals, Berlin, Germany, 13-17 October 1997 and the WHO Consultation on the use of Quinolones in Food Animals and Potential Impact on Human Health, Geneva, Switzerland, 2-5 June 1998. The Global Principles strengthened and endorsed earlier WHO recommendations and recognised that all stakeholders concerned with the use of antimicrobials in both food animals and humans must be involved in an overarching global strategy. More than 30 national and international review documents were considered in the production of the Global Principles.

The Global Principles described a number of areas relating directly to surveillance of antimicrobial resistance in food and food animals, and these may be summarised as follows:

- Data generated from the surveillance of antimicrobial resistance and antimicrobial usage should play a key role in the development of national policies for the containment of antimicrobial resistance. These data are also essential in the pre- and post-licensing process and in the development of treatment guidelines for veterinary use.
- Programmes to monitor antimicrobial resistance in animal pathogens, zoonotic agents (for example, *Salmonella* spp. and *Campylobacter* spp.), and bacteria known to be indicators of antimicrobial resistance (for example, *Escherichia coli* and *Enterococcus faecium*) should be implemented to cover bacteria recovered from animals, food of animal origin and humans. Veterinarians, medical doctors, authorities and other stakeholders should be kept regularly informed about the surveillance results and trends. Antimicrobial susceptibility testing should be performed according to standardized methods using appropriate quality control, and be reported quantitatively to allow comparison of results.
- The pre-licensing safety evaluation of veterinary antimicrobials should include consideration of potential resistance to human drugs. Decisions regarding registration of antimicrobials for use in food animals should be based on scientific data and, unless otherwise justified, should include the potential rate and extent of resistance in relevant bacteria associated with the proposed use in food animals in the pre-approval evaluation.

- There should be monitoring of resistance in bacteria from food and food animals to identify emerging health problems and timely corrective actions to protect human health.
- The authorisation of veterinary antimicrobial products should take account of data on antimicrobial resistance among relevant bacterial strains and should ensure that recommended dosages are optimal for therapy, taking into consideration pharmacokinetics, clinical efficacy, residues, and, if available, other relevant data in order to minimize the development of resistance.
- Existing product labelling should also be reviewed, when necessary, by regulatory authorities to ensure that the recommended dose and duration of use are consistent with current knowledge of efficacy, antimicrobial resistance, pharmacokinetics, pharmacodynamics and prudent use.
- A risk-based evaluation of the potential human health effects of all uses of antimicrobial drugs in food-producing animals should be conducted. Those antimicrobials judged to be essential for human medicine should be restricted and their use in food animals should be justified by culture and susceptibility results.
- Post-approval surveillance is indispensable and surveillance of resistance to antimicrobials belonging to classes considered important in human medicine should be closely monitored so as to be able to detect emergence of antimicrobial resistance in time to allow corrective strategies to be implemented as part of an efficient post-licensing review.
- Post-approval surveillance of antimicrobial resistance should include identification of the appropriate bacteria and methods of collection. Relevant antimicrobials to be included in such post-approval surveillance programmes should be guided by a risk-based priority list under the direction of the relevant authority. The methods and data should be made publicly available. Such surveillance may be carried out with the participation of the veterinary pharmaceutical industry.
- Where resistance increases above levels of concern, epidemiological and/or experimental investigations to identify risk factors may be needed and proportionally incremental mitigation strategies, such as education, infection control, labelling changes, changes in dosing and duration of use, should then be implemented.
- Locally-derived species-specific veterinary treatment guidelines should include a list of antimicrobials for conditions commonly presenting in clinical practice and offer a rational treatment choice based on scientific data and knowledge, the disease and resistance situation, practical experience and human health concerns. If several antimicrobials can be used, guidelines should make recommendations on different antimicrobials to be used. However, the clinical experience and judgment of the practitioner should determine the final choice.
- The responsibilities of veterinarians and/or producers include that, for each treated animal or group of animals a health record should be kept to support the choice of empirical therapy. The record should include data on antimicrobial use, previous antimicrobial susceptibility test results; and/or previous treatment outcomes.

- Veterinarians should continuously evaluate their prescribing practices. This would be based on information such as the main indications and types of antimicrobials used in different animal species and appraisal in relation to available data on antimicrobial resistance and current use guidelines.

The Codex Alimentarius Commission of the FAO has also produced several discussion papers and other documents that are relevant to the surveillance of antimicrobial resistance in bacteria from food and food animals. The Codex Committee on Residues of Veterinary Drugs in Foods produced a discussion paper on antimicrobial resistance and the use of antimicrobials in animal production (CX/RVDF 01/10), including a proposed draft code of practice to minimise and contain antimicrobial resistance. Several of these points were further expanded in the Codex Alimentarius Commission proposed draft code of practice to minimise and contain antimicrobial resistance of November 2002 (CX/RVDF 03/6). Included in these documents were the following recommendations relating to surveillance for antimicrobial resistance in food and food animals:

- Responsible use of antimicrobials should be based on the results of resistance surveillance and monitoring. There should be a structured approach to the investigation and reporting of the incidence and prevalence of bacterial resistance. National surveillance of animal bacteria resistant to antimicrobial agents is recommended. The relevant authorities should implement this programme, established with the results of risk analysis, which makes it possible to rank priorities regarding antimicrobials and animal bacteria, whether or not they are pathogenic for man. The methods used to establish such programmes should be harmonised as much as possible at the international level and reference is made in the documents to the OIE guidelines on surveillance programmes and laboratory methods.
- Pharmacovigilance programmes – regulatory authorities should have in place a pharmacovigilance programme for the monitoring, reporting and recording of adverse reactions to antimicrobials, including the lack of efficacy related to resistance. Information collected through the pharmacovigilance programme should form part of a comprehensive strategy to minimise antimicrobial resistance.
- Targeted Surveillance – A surveillance programme to assess the impact of use of antimicrobial agents, especially those that are intensively used, on the selection of antimicrobial resistant bacteria in food producing animals may be implemented after the granting of the marketing authorisation. In certain cases, the surveillance programme should evaluate not only resistance development in target animal pathogens, but also in foodborne pathogens and/ or commensals.
- Assessment of the potential of (new) antimicrobials to select for resistant bacteria may include examination of the pre-existing level of resistance, in pathogens of human health concern (baseline determination).
- Specific surveillance should be directed by priorities (regarding antimicrobials and bacterial species) established by risk assessment.
- Susceptibility data should be used to establish therapeutic guidelines. Susceptibility data from individual units or farms may also be used to establish local guidelines.

The Codex Alimentarius document entitled “Risk profile on antimicrobial resistant bacteria in food” (August 2001 CX/FH 01/12), commented on the sources of food-borne pathogens from foods and stated that in the developed countries, humans usually acquire *Salmonella* and *Campylobacter* through ingestion of foods of animal origin that are contaminated with animal faecal material, but a wide variety of other foods can be involved including water, fruits and vegetables. There were regional differences in the most common food sources of these bacteria due to variations in the prevalence of these bacteria in the food chain and food consumption patterns. Foods may cross-contaminate other food items or food plant during preparation / processing, therefore a large number of foods can potentially act as sources of bacteria that are resistant to antimicrobials.

This document also commented on the food-borne pathogens with a human reservoir, namely, *Salmonella* Typhi, *Shigella* spp. and *Vibrio cholerae* (which also has an environmental reservoir) in which increasing antimicrobial resistance is also of concern. Humans usually acquire these bacteria through ingestion of foods, including water, which are contaminated with human faeces. Use of antimicrobials in humans is the principal source of antimicrobial resistance among these bacteria, which are seen mainly in the developing world (except some species of *Shigella*). The primary point at which food is contaminated with animal faeces is at slaughter; food handling up to retail provides the point at which food may be contaminated with human faecal bacteria or cross-contaminated with animal faecal or environmental bacteria.

The document states that the public health risk of antimicrobial use in aquaculture and horticulture has not been well characterised but should be evaluated. There is a specific need for generating data addressing such issues. Use of disinfectants and some food preservation additives may also need to be considered and can affect the prevalence of antimicrobial resistant bacteria in foods. The document advises that the regular monitoring of antimicrobial susceptibility in bacteria from food animals should be conducted and that there was also a recommendation for surveillance of the antimicrobial susceptibility of bacteria in human and animal waste slurry.

The report recommends that resistance to antimicrobials that poses a potential human health threat concern should be monitored with appropriate surveillance systems after approval of the antimicrobial in question. Monitoring programmes should permit the identification of trends and could be a useful tool for estimating the magnitude of the resistance concern and the need for any re-evaluation.

In addition to surveillance for bacterial resistance, prevalence studies of food-borne pathogens and other organisms of significance, as well as collection of information relating to prevalence of bacterial organisms in feed and water were important factors to consider in control strategies. The type of husbandry or production system involved might have important bearings on the propensity of resistant organisms to spread and should also be recorded.

An OIE Regional Commission for Europe study in 1997 revealed that few countries had established official resistance monitoring programmes, that risk analysis was not commonly applied and that there were very different approaches and methodologies in the European Region. The study showed that additional efforts should be made to develop official antimicrobial resistance surveillance/ monitoring programmes and to improve the harmonisation of laboratory methodologies, which in turn would improve the reliability and comparability of generated resistance data. Two conferences on

antimicrobial resistance were held by the OIE in 1999 and 2001 and in 2000 the OIE appointed an expert group on antimicrobial resistance which established 5 guidelines relating to this topic. OIE Guideline Two describes the standardisation and harmonisation of laboratory methodologies for the detection and quantification of antimicrobial resistance and OIE Guideline One the harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and animal-derived foods. Four of these guidelines were adopted during the last annual session of the OIE in May 2003 and are now included in the OIE Terrestrial Animal Health Code (White et al. 2001, Franklin et al. 2001). The objective of Guideline One is to allow generation of comparable data from various national surveillance and monitoring systems in order to compare the situations in different regions or countries and to consolidate results at the national, regional and international level. It provides definitions of surveillance and monitoring and stipulates that national systems should be able to detect the emergence of resistance and to determine the prevalence of resistant bacteria. The resulting data should be used in the assessment of risks to public health and should contribute to the establishment of a risk management policy. Specific factors identified for harmonisation include the animal species, food commodities, sampling plans, bacterial species, antimicrobials to be tested, laboratory methods, data reporting, database structure and the structure of reports. The OIE Guidelines are further discussed in section “Surveillance for antimicrobial resistance”.

Several other national and international bodies have addressed the issue of surveillance of food, food animals and other sources for resistant bacteria and many of these were included in the review of documents that took place when the WHO Global Principles were devised. For example, in 1995 an American Society of Microbiology report advocated a significant increase in resistance monitoring in the US. In 1999, the Scientific Steering Committee of the European Commission made four primary recommendations relating to the use of antimicrobial growth promoters, one of which was that effects of interventions should be monitored and evaluated. The Arbao (Antibiotic resistance in bacteria of animal origin) EU-funded European concerted action made a number of recommendations (Arbao, 2002) regarding surveillance procedures, the majority of which have been incorporated into the OIE guidelines. More recently, EU legislation adopted on September 29, 2003 lays down requirements regarding control and monitoring of various zoonoses, including monitoring of antimicrobial resistance in zoonotic agents as well as other relevant bacteria if required, such as indicator organisms. The legislation requires member states to ensure that monitoring provides comparable data on the occurrence of antimicrobial and that such monitoring should supplement the monitoring of human isolates conducted under other directives. The EU legislation has incorporated most of the OIE monitoring recommendations. www.health.fgov.be/WHI3/krant/krantarch2003/kranttekstsept3/030930m12eu.htm

B. Surveillance for Antimicrobial Resistance.

Objectives

Surveillance for antimicrobial resistance in bacteria from animals, food and other sources may have the following objectives:

- To follow trends in existing resistance in bacterial strains (or establish baseline resistance levels in those strains).
- To detect newly-emerging resistance in bacterial strains.

- To highlight existing or emerging resistance problems and direct appropriate research into those areas.
- To optimise the choice of antimicrobials used in therapy.
- To monitor the effects of interventions.
- To provide data necessary for conducting risk analyses to determine risk to human and animal health.
- To provide data to inform policy decisions.
- To assess the level of cross-contamination between foods (eg carcasses at and after slaughter).
- To assess the degree of contamination of food (eg. carcasses or meat after slaughter) from other sources, including residual carry-over, human processors, plant, other environmental sources.
- To identify critical control points and assess the transfer to foods of hazards detected on farm or in the environment.

Methodology

The methods appropriate for surveillance of antimicrobial resistance in bacteria from foods and food animals have been reviewed by the OIE and their recommendations are summarised here.

Animal species and food to be included in monitoring

Each OIE Member Country should examine its livestock production systems and decide, after risk analysis, the relative importance of antimicrobial resistance for animal and human health. Categories of livestock that should be considered for sampling include cattle and calves, slaughter pigs, broiler chickens, layer hens and/or other poultry and farmed fish.

Food of animal origin that should be sampled may also be determined after risk analysis. Plants and vegetables of different types may have been treated with certain antimicrobials in some cases or may have been exposed to manure or sewage from livestock and can therefore become contaminated with resistant bacteria of animal origin. The recent EU legislation will recommend that cattle, pigs and poultry (beef, pork, broiler, turkey meat) and table eggs should be sampled and that domestically-produced and imported food should be examined for each category.

Animal feed, including imported feed, may also be considered in monitoring and surveillance programmes.

Bacterial species and/ or strains to be included in monitoring

Bacteria recovered from animals may be subdivided into three classes, zoonotic organisms, indicator bacteria and animal pathogens. Zoonotic bacteria should include *Salmonella* spp. (which in turn should include at least *S.Typhimurium* and *S. Enteritidis* – the selection of other relevant serovars to be dependent on the epidemiological situation in each country), *Campylobacter jejuni* and *C.coli* and *E.coli* O157 or VTEC. Indicator organisms comprise *E.coli* and *E. faecium/ faecalis* and these should be isolated from healthy animals at the point of slaughter. Monitoring of resistance in animal pathogens is important to detect emerging resistance that may pose a concern for human and animal health and to guide veterinarians in their prescribing decisions. Information on the occurrence of antimicrobial

resistance in animal pathogens is in general derived from routine clinical material sent to veterinary diagnostic laboratories. These samples, often derived from severe or recurrent clinical cases including therapy failures, may provide an over-estimate of the average levels of resistance. Animal pathogens that may be considered in monitoring are shown in Table 1, which is taken from the OIE Guidelines.

Sampling Strategy used in the monitoring

Monitoring should provide data that can be used to fulfill the objectives described at “Objectives”. Within countries, comparison between levels of resistance in non-human and human sources may be a primary objective and in these cases, harmonisation between the medical, veterinary and food susceptibility testing procedures in those countries will be a priority to enable direct comparison of national results. Where international comparison of levels of resistance is desired, then the priority may be to use an internationally agreed standard method that will facilitate the desired international comparison. Even in cases where susceptibility testing has not been harmonised, then data may still be useful to assess temporal trends of increasing or decreasing levels of resistance that may tie in with similar trends in other populations of organisms.

Active monitoring programmes (i.e. programmes specifically designed to address the study objectives and which actively acquire isolates) should be considered for zoonotic agents (particularly *Campylobacter*) and for all indicator bacteria and these in general rely on statistical sampling of the animal population at the point of slaughter in abattoirs. Where zoonotic agents are at a low prevalence (e.g. *Salmonella* in many countries), then passive monitoring (reliance on clinical veterinary isolates) may be the most cost-effective way of obtaining sufficient numbers of isolates and is particularly appropriate in farmed animal species in which *Salmonella* causes clinical disease. There are various hybrid permutations that can be devised between these two approaches, for example serological monitoring of pig herds may reveal positive herds which are then sampled for *Salmonella*, with the *Salmonella* isolates recovered then entering the monitoring programme. Representative sampling should be ensured and results should reflect population trends rather than differences in the intensity of sampling. The latter may be overcome by various approaches, including for example testing only the first isolate of a particular *Salmonella* serotype recovered from a single location in any 3 month or year-long period. Where the organisms in question are under ongoing selective pressure, then a 3 month sampling time frame may be preferred to assess emergent resistance. Procedures will also need to take account of any biases that may be introduced if sentinel sub-populations are monitored. Sampling protocols are also likely to reflect any control or contingency measures that will be instituted if resistance is detected. If selected sub-populations of organisms are being examined from sentinel regions, then these may detect the resistance later than programmes that test all isolates from a geographical region. It would not be appropriate to link this sentinel surveillance at selected sites to immediate control measures at particular premises as resistance (when detected) is likely to have spread to other premises falling outside the sentinel monitoring. Thus, the level of monitoring that is put in place may reflect the controls that will be instituted if a particular type of resistance is detected.

Sample Sizes

The OIE Guidelines recommend that sample size should be large enough to avoid failure to detect existing resistance, but not excessively large to avoid the waste of resources. Sampling should be conducted on a statistical basis, particularly for indicator organisms and common zoonotic organisms such as *Campylobacter*; however, statistically-based sampling may not be practicable for countries with, for example, a low incidence of *Salmonella* in animals at slaughter. The sampling strategy should

ensure that the sample is representative of the population of interest. Numbers of isolates tested should be sufficient to meet the following objectives:

- detection of the occurrence of resistance patterns.
- estimation of the prevalence of resistance to an antimicrobial substance.
- assessment of changes in the prevalence rate.

The Arbao Concerted Action identified that sampling strategy and sample size will depend on the objectives of the surveillance, namely alert (to detect new occurrence of resistance), determination of the prevalence of resistance, comparison of prevalence in different regions or groups or production types, or determination of trends in the emergence or decline of resistance.

For a given sample size, statistical tables can be used to give the probability of isolating (or not isolating) a resistant strain where it is important to detect new occurrence of resistance (alert). For example, the sample size required to detect a 1% level of resistance in a bacterial population with a probability of 0.90 is 44 bacterial isolates, with a probability of 0.95 is 90 bacterial isolates and with a probability of 0.99 is 459 bacterial isolates.

The EU Directive mentions a target figure for surveillance of 385 bacterial isolates, per year per bacteria and per animal species (assuming a 50% prevalence of resistance, to obtain an estimate of the level of resistance with desired confidence intervals of $\pm 5\%$ and precision of 95% will require 385 isolates). Higher and lower prevalences of resistance to be assessed with the same confidence interval need fewer isolates. [The number of samples actually required must be multiplied by the occurrence of the bacterial strains in the primary samples]. OIE Guideline One contains a statistical table summarising similar data and has a value of 384 isolates to give the same level of confidence and precision. If there is an assumed 5% prevalence of resistance, then 73 bacterial isolates will need to be tested to determine the percentage of resistance with a confidence interval of $\pm 5\%$.

Zoonotic and indicator bacteria can most easily be collected at the slaughterhouse and selected on a statistical basis. Healthy animals are sampled and there should be one isolate per animal group to avoid clustering. Faecal samples may be collected from cattle and pigs and caeca from poultry.

Food should be sampled at retail to reflect most closely the risks to consumers.

Harmonisation of Laboratory Methodology

OIE Guideline Two provides rules and protocols for susceptibility testing methodologies to be used by OIE Member Countries and advises on procedures to initiate standardisation and harmonisation of test methods and also to initiate standardisation and harmonisation of the interpretation of antimicrobial susceptibility test results.

Bacterial identification may be of particular importance when interpreting susceptibility data since some bacteria are intrinsically resistant to certain antimicrobials; ISO standards for bacterial identification in food and feed are available for *Salmonella* and enterobacteriaceae. This is particularly important when examining resistance in *E.faecium* and *E.faecalis*.

Quantitative data should be recorded (either zone diameters from disc diffusion tests or minimum inhibitory concentration values), since this assists in comparing populations of organisms and can circumvent potential problems that arise where different breakpoints are used. Harmonisation of breakpoint interpretive criteria is preferable, but may not be currently achievable between all countries. Regardless of the susceptibility testing method used, the procedures must be standardised to ensure accurate and reproducible results, and appropriate quality control reference organisms need to be tested every time susceptibility testing is performed in order to ensure accuracy of the data.

OIE Guideline Two describes how antimicrobial susceptibility breakpoints are established by national professional societies or regulatory agencies, but mentions that there can be notable differences in breakpoints among different countries for the same antimicrobial agent. The development of a concept known as ‘microbiological breakpoints’, which is based on the population distributions of the specific bacterial species tested, may be more appropriate for antimicrobial surveillance programmes. In this case, bacterial isolates that deviate from the normal susceptible population are designated as resistant, and shifts in susceptibility to the specific antimicrobial/bacterium combination can be monitored.

Reports

The EU Directive will recommend that reports contain sections describing materials and methods, data, discussion and comments (trends, emerging resistance), additional information (such as changes in drug licensing) and data on animal husbandry activities. The data section should include occurrence of resistance patterns, estimation of the prevalence of resistance to an antimicrobial and changes in prevalence rate.

Continuous Surveillance Programmes in Food Animals

Within the scope of this document, it is not possible to discuss all monitoring programmes that are currently in place worldwide and only a few selected examples will be mentioned, with particular emphasis on monitoring in certain Nordic countries.

DANMAP (the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme) was established in 1995 and provides susceptibility data on representative bacterial isolates from both healthy and diseased animals. It also provides data on antimicrobial consumption, linked data on the antimicrobial susceptibility of bacteria from man and demographic data on the numbers of farmed livestock in Denmark. The veterinary pathogens included in 2001 were *E.coli* serotypes O2 and O78 from poultry (n=19), F5 from calves (n=86) and O149 from weaned pigs (n=196), *Staphylococcus aureus* (n=60) and coagulase-negative Staphylococci (n=59) from cattle and *Staphylococcus hyicus* from pigs (n=53). Considering zoonotic organisms, DANMAP 2001 provided susceptibility data on *Salmonella* isolates from food animals (generally clinical infections in cattle and subclinical infections in pigs and poultry), with one isolate of each serotype being included for each farm premises, except in pigs where isolates were randomly selected from all isolates serotyped. The report provides resistance data on *Salmonella* isolates from imported food and generally concentrates on resistance data for serotypes Typhimurium and Enteritidis, with less discussion of other serotypes. Susceptibility data for *Campylobacter jejuni* from cattle (n=38), broilers (n=79), broiler meat (n=74) and turkey meat (n=43) is reported in the DANMAP 2001 report and comparisons are made between resistance levels in Danish

food animals, Danish food, imported food (collected at retail) and domestically-acquired and travel-associated human cases. *Campylobacter coli* from broilers (n=12) and pigs (n=94) are also included in the monitoring, with all *Campylobacter* and indicator organisms being recovered from healthy animals at slaughter. *E.faecium* were recovered from broilers (n=131), cattle (n=18) and pigs (n=175) and *E.faecalis* from broilers (n=82) and pigs (n=184). *E.faecium* isolates were also sampled from turkey meat (n=21), broiler meat (n=44), beef (n=45) and pork (n=16) as were approximately similar numbers of *E.faecalis* isolates. Indicator *E.coli* were sampled from broilers (n=134), cattle (n=85), pigs (n=304), beef (n=94), pork (n=48), broiler meat (n=122) and other poultry meat (n=98). Resistance levels in *E.coli*, *E.faecium* and *E.faecalis* in Danish food animals, Danish food and imported food are compared.

The NORM-VET programme (Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway) includes data on animal population statistics, and consumption of antimicrobial agents. Reports also provide linked data to antimicrobial resistance in human bacteria collected over the same period. Animal clinical isolates include *E.coli* from pigs with enteritis (n=39) and from cases of septicaemia in broilers (n=52). *E.coli*, *E. faecium* and *E. faecalis* from healthy pigs and broilers are sampled as indicator organisms both in faeces and meat collected at abattoirs and sampling is proportional at each abattoir relative to the total number of animals slaughtered in Norway. The maximum number of meat or faecal samples collected for either of these purposes is 212. All *Salmonella* isolates (n=73) recovered from animals and feed mills and seagulls in Norway in 2002 were included in the susceptibility testing programme. There is a Norwegian Action Plan against *Campylobacter* spp. in broilers and all flocks slaughtered before 50 days are tested for the presence of *Campylobacter*. A single isolate from each positive farm (n=122) as well as isolates from broiler meat (n=39) were tested in the susceptibility programme in 2002.

The Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) programme in common with all three reports from the Nordic countries provides data on antimicrobial consumption and is linked to data on the susceptibility of bacteria recovered from man. *Salmonella* infection in farm animals is notifiable in Sweden and one isolate from each incident is included in annual monitoring. *Campylobacter* spp. isolates (n=100) are obtained from healthy broiler chickens sampled at slaughter for susceptibility testing and of these 84 proved to be *Campylobacter jejuni* in 2002. For indicator bacteria, the SVARM programme does not sample all species every year, but rotates the farmed species that are sampled. Thus, in 2000 indicator *E.coli* from chickens (n=274) and cattle (n=293) were sampled, whereas in 2001 chickens (n=296) and pigs (n=308) were sampled. A similar rotation of sampling has been done for *Enterococcus* spp. isolates from cattle, chickens and pigs with SVARM 2002 reporting susceptibility results for *E.faecalis* (n=57), *E.faecium* (n=189) and *E.hirae* (n=45). Veterinary pathogens collected from all regions of Sweden under the SVARM programme in 2002 include *E.coli* (n=340) and *Brachyspira hyodysenteriae* (n=109) from pigs and *E.coli* (n=220 over the period 1992-2002) *S.aureus* (n=100), *Streptococcus dysgalactiae* (n=100) and *Streptococcus uberis* (n=98) from cattle. SVARM 2002 also includes data on resistance in *Streptococcus zooepidemicus* (n=163) and *E.coli* (n=166) from horses and on *E.coli* (n=204) from canine urine and *Staphylococcus intermedius* (n=133) recovered from canine skin. Data from *E.coli* recovered from feline urine (n=46) are also included. SVARM also provides demographic data for the major farmed animal species in Sweden.

In summary, the reports from Denmark, Norway and Sweden:

- Include demographic details.

- Provide quantitative data on the susceptibility of animal pathogens, zoonotic and indicator organisms.
- Include data on the consumption of antimicrobials.
- Also include (or are combined with reports concerning) data on susceptibility of human bacteria, though the extent to which medical and veterinary harmonisation of susceptibility testing has occurred and the extent to which integration of the medical and veterinary parts of the reports have occurred is variable.
- The reports from Norway and Denmark include data on susceptibility of food isolates, with the data from Denmark also discriminating between imported and home-produced food. The food isolates in the Danish monitoring are collected at retail, whereas in Norway are collected from meat at abattoirs.

In France, the RESABO network collects susceptibility data on bovine pathogens that have been tested at a number of different laboratories and this is co-ordinated by the Agence Française de Sécurité Sanitaire des Aliments (AFSSA). The RESABO network has collected susceptibility data on bovine pathogens for over 20 years and in 2001 this network was extended to include bacteria from pigs and poultry and re-named RESAPATH. AFSSA also co-ordinate an extensive susceptibility surveillance programme on *Salmonella* which in 1998 tested the susceptibility of 6059 *Salmonella* serotypes, originating from human food, animal feed, food and breeding animals or the environment. Isolates are collected from different laboratories and tested centrally in this extensive and comprehensive monitoring system. Results of monitoring are published annually by AFSSA.

The UK has an extensive system for monitoring resistance in a wide range of veterinary pathogens, with more than 11,000 organisms tested in 2002. A network of 14 regional veterinary diagnostic laboratories contribute data to this programme, which is published annually on the Defra (Department of Environment, Food and Rural Affairs) web-site and includes data for a range of veterinary organisms not included in many other reports. Complementing this surveillance, resistance in zoonotic and indicator organisms in cattle, sheep and pigs is monitored in statistically-based surveillance at abattoirs, though poultry have not yet been included in the UK abattoir surveillance programme. Results are available on the web at www.defra.gov.uk/animalh/diseases/zoonoses/index.htm

In the USA, the National Antimicrobial Resistance Monitoring System was instigated in 1996 to monitor changes in the antimicrobial susceptibility of non-typhoidal *Salmonella* spp. in human and animal diagnostic specimens, healthy animals and raw animal-derived products collected from federally-inspected slaughter and processing plants. In 1999, 8,508 *Salmonella* isolates of animal origin were tested. In 2001, the susceptibility of *E.coli*, *Campylobacter* spp. and *Enterococcus* spp. was additionally monitored in humans and animals and the programme now includes the testing of retail isolates of meat. Results of this programme are available at www.arru.saa.ars.usda.gov.

Other monitoring networks include the VAV (Vigilancia Antibiorresistencias Veterinaria) network in Spain for monitoring antimicrobial resistance in bacteria from animals and man which was created in 1996. The monitoring of resistance in *E.coli* and *E. faecium* from healthy pigs began in 1998 and collection of data from *Salmonella* isolated from poultry at slaughterhouses in 1999. An analysis of national monitoring programmes in a number of European countries was recently completed (Wray and Gnanou, 2000).

The European Animal Health Study Centre (CEESA) performed surveillance for antimicrobial resistance in chickens, pigs (over 1999-2000) and cattle (over 2000-2001) in four countries per host and examined *E.coli* (n=2118), *Salmonella* spp. (n=271) and *Campylobacter* spp. (n=1326) recovered from these animal species. The study used standardised isolation methods and all determinations of minimum inhibitory concentration were performed at a single laboratory to maximise comparability of results (Bywater et al. 2003).

Surveillance Programmes in Food

The surveillance data relating to antimicrobial resistance of foodborne bacteria isolated from foods is variable in extent and quality. Often, surveys of foods have been performed on an occasional basis, rather than complementing the ongoing surveillance studies performed in animals and man or being repeated periodically with consistent methodology to assess trends. Furthermore, surveys of foods may have been designed to address specific questions that were of particular importance at that time. For example, in the 1990's several studies were instigated looking at the prevalence of vancomycin-resistant enterococci in foods. Details of the origins of foods are important, because levels and types of resistance are often different in different geographical locations and some studies have been specifically designed to investigate such differences in home-produced and imported foods – for example the DANMAP programme. The major organisms to be surveyed in food include *Salmonella*, *Campylobacter*, *E.coli* (including O157) whilst other organisms of relevance include *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Staphylococcus aureus* and *Enterococcus* spp. Harmonisation of methods with veterinary and human susceptibility testing procedures is essential to maximise the usefulness of the data obtained.

Surveillance Programmes in Companion and Other Animals

Surveillance of antimicrobial resistance in bacteria recovered from companion animals has generally been in the form of ad hoc surveys of samples of bacterial isolates from specific locations or of reviews of veterinary clinical data gathered over a period of time at specific locations. The exception to this are the surveillance programmes in Norway and Sweden, which have been described in section “Continuous surveillance programmes in food animals”.

C. Occurrence of resistance and trends over the last few decades in selected organisms

***Salmonella* spp.**

- Trend to emergence and later decline of particular serotypes (particular definitive types of *Salmonella* Typhimurium), most of which are multiply-resistant to antimicrobials.
- Increasing emergence of resistance to fluoroquinolones.
- Increasing emergence of resistance to third generation cephalosporins.
- Unknown why resistance develops in some serotypes, yet is rare in others.
- Dissemination of particular resistant clones because of particular husbandry procedures or the structure of the farming industry can greatly influence resistance prevalence figures.

Salmonella serotypes tend to emerge and then decline in prevalence over time and many of these emergent strains have in recent decades shown multiple antimicrobial resistance. In the 1960's *S.Typhimurium* DT29 emerged in the UK which was resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, sulphonamide, tetracycline and furazolidone. This became uncommon in the 1970's and was replaced with DT's 204, 193 and 204c. The latter dominated as the main phage type until the 1990's when it declined and after a short period, *S.Typhimurium* DT 104 emerged as the most common definitive type. Resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracyclines increased in *S.Typhimurium* from 1994 and this was related to the spread of DT 104. The reasons for the emergence of particular phage types and for their eventual decline are unknown.

The other main trend of importance in *Salmonella* is related to development of resistance to two of the compounds used for treatment of human salmonellosis – the fluoroquinolones and third generation cephalosporins. A temporal association has been shown between the authorisation of fluoroquinolones for animal treatment in the UK (and other countries) and an increase in resistance to nalidixic acid in *Salmonella* Typhimurium isolates from farm animals. Detection of a novel gyrase mutation allowed the elegant demonstration of spread *S. Typhimurium* that was resistant to nalidixic acid from pigs to man, via the abattoir, in Denmark (Mølbak et al. 1999).

Third generation cephalosporins are particularly important for the treatment of salmonellosis in children in many countries. Resistance to these compounds in *Salmonella* isolates from Europe is rare, though is much more common in other parts of the world. In North America, multi-drug resistant *Salmonella* Newport (resistant to third generation cephalosporins and other antimicrobials) has recently caused significant human and animal disease. Resistance in North American *Salmonella* Newport isolates is due to the possession of the *cmv2* gene on a plasmid (this gene encodes a beta-lactamase that is normally present on the chromosome of wild-type isolates of *Citrobacter freundii*), whilst in South America, CTX-M beta-lactamase enzymes have been detected in *Salmonella* isolates.

***Campylobacter* spp.**

- Increasing fluoroquinolone resistance in *Campylobacter jejuni* has been observed in many countries worldwide (Engberg et al 2001).
- Macrolide resistance tends to be high in *Campylobacter coli* isolates recovered from pigs in many parts of Europe.

Escherichia coli

E.coli O157 isolates from the UK rarely show multiple resistance (defined as resistance to four or more unrelated antimicrobials), but resistance to streptomycin, sulphonamides and tetracyclines increased during the 1990's (Willshaw et al. 1996). Recently, *E.coli* O157 has been detected in the USA with resistance to third generation cephalosporins (data available at www.arsu.saa.ars.usda.gov).

Monitoring in Denmark of resistance in indicator *E.coli* from animals at slaughter, showed that resistance levels have remained relatively unchanged over the period 2000-2001, as did levels of resistance from a variety of foods (DANMAP 2001). Levels of resistance in *E.coli* from cases of mastitis in dairy cows in the UK have been remarkably stable over a five year period of monitoring (www.defra.gov.uk/animalh/diseases/zoonoses/index.htm).

Listeria monocytogenes

Recent studies have demonstrated that *Listeria monocytogenes* can acquire tetracycline resistance genes from the commensal intestinal enterococcal flora and that low level tetracycline can be an important selective pressure to direct this transfer (Charpentier and Courvalin 1999).

Yersinia enterocolitica

Danish studies in 1996 showed that resistance (other than intrinsic resistance to ampicillin) was low. (Anon, 1996)

Enterococci

Overall, there has been a good association recorded in Denmark between the phasing out of antimicrobial growth promoters and declining resistance trends in *E.faecium* from broilers and pigs as well as from broiler meat and pork. Where the trend has not been as rapid as expected, linkage of resistance genes to genes for other antimicrobials that are still administered therapeutically or to heavy metals such as copper, appear to have been responsible (i.e. co-selection is occurring) (DANMAP 2001). The Danish studies have also highlighted the importance of colonisation of processing equipment with particular clones of *E.faecium* that are able to persist and contaminate food. However, Norwegian data (NORM-VET 2002) has shown that vancomycin-resistant enterococci can still be detected in 91% of faecal samples from Norwegian broilers seven years after the ban on use of avoparcin. The apparent discrepancy in findings reflects test procedures, with selective enrichment being used in the Norwegian studies, whereas the Danish studies test randomly selected isolates from primary (non-selective) culture plates.

D. Monitoring of resistance genes

In future, susceptibility monitoring may need to be driven by the spread of particular resistance determinants rather than by monitoring bacterial species. For example, in North America, the *cmy2* gene conferring resistance to third generation cephalosporins, was initially found on a plasmid in strains of *Salmonella* Newport, but has been detected more recently in *Salmonella* serotypes Agona and Heidelberg and in *E.coli* O157. This spread of particular transferable genetic elements bearing particular resistance genes may be particularly important in the case of the transferable resistance to fluoroquinolones that has been described and for resistance to carbapenem and imipenem compounds.

E. Harmonisation Programmes Underway

Harmonisation programmes currently underway include several programmes dealing exclusively with bacteria of human origin at the European or global level. WHO have set up the Global Salm-Surv network that is monitoring serotypes and antimicrobial resistance in *Salmonella* globally and the Enternet programme is performing a similar function at the European level. There is an orientation towards isolates from medical sources in both these programmes. Within the EU, the Community Reference Laboratory for *Salmonella* at Bilthoven has organised ring trials to compare methods and procedures in national *Salmonella* reference laboratories. The Arbao II network, building on OIE Guidelines and the conclusions of Arbao I, will run for three years and commenced in January 2003.

This will aim to harmonise susceptibility testing procedures throughout the EU by circulation of control strains from a range of organisms in ring trials. Data from participating countries will also be collected, collated and interpreted taking into account the results obtained from the circulated control strains. For some organisms, there are no internationally accepted control strains or standardised test procedures available (e.g. *Campylobacter*, *Brachyspira hyodysenteriae*). A network of five European veterinary laboratories recently agreed to set up an exercise to generate validated control strains for *Brachyspira*.

Therefore the position may be summarised as follows:

- Common standardised susceptibility testing procedures are not currently in use globally.
- Ring trials for some organisms have been set up to try to harmonise procedures.
- Programmes to establish well-defined control strains for a few organisms are underway and will help to harmonise procedures.
- There are no internationally accepted standardised test procedures available for some organisms.
- There is no global harmonisation of breakpoints.

F. Analysis - uncertainties that still exist, current discrepancies between groups

The most developed and integrated system for surveillance of antimicrobial resistance in humans, food animals and retail food is that of Denmark. However, even in this programme, there are only relatively low numbers of isolates tested for some bacterial species from some matrices. This is a feature of many other surveillance systems and the number of isolates for several bacterial species currently tested in many other European monitoring programmes does not approach the target of 385 recommended in the recent EU Zoonoses Directive.

Three broadly differing approaches can be identified in the antimicrobial susceptibility monitoring systems of countries globally. The first approach is to test all representative bacterial isolates with a technique that will determine minimum inhibitory concentration (MIC). The second approach is to select a proportion of the total available isolates and to test these with a technique that will determine the MIC. The third is to screen a larger number of isolates (or all isolates) with a cheaper susceptibility test procedure, such as disc diffusion and then, when considered necessary, select a sub-population of these organisms showing potential resistance of interest for examination by a technique which will determine the MIC. A number of permutations of these three approaches exist. One advantage of the third approach may be that a much larger number of isolates can be screened with available resources.

The approaches to sampling also need careful consideration in relation to potential control measures, in particular whether a sub-sample of the population or the entire population (or all available bacterial isolates) will be tested. Contingency or action plans for dealing with emergent or increasing resistance might relate to the first detected resistant isolate or alternatively, containment of existing resistance levels and the sampling approach will differ in each case. It would be appropriate to sample a representative subset of the population for assessment of resistance levels and then apply general controls, but reliance on this approach to detect and control initial emergent resistant isolates on individual premises would not be appropriate.

Phenotypic characterisation is insufficient on occasion for some important types of resistance and further extended phenotypic characterisation or genetic characterisation may be required to fully determine the significance of findings. This may be particularly true in the case of resistance to fluoroquinolones (where there are reports of transferable resistance emerging), for third generation cephalosporin resistance and for carbapenem/ imipenem resistance. For example, a range of beta-lactam compounds may be needed in surveillance programmes to assess the potential significance of third generation cephalosporin resistance in enterobacteriaceae. The role of cross-resistance in encouraging the spread of resistance genes (that have originated in other ecological niches) within the farm or aquaculture environment needs to be taken into account. For example, the ampicillin resistance gene in *Salmonella* Typhimurium DT 104 BLA_{CARB} confers resistance to carbenicillin, an antimicrobial that has never reportedly been used in agriculture. Because the resistance gene also confers resistance to ampicillin, use of ampicillin in agriculture can select for its spread. Similar considerations apply to many of the third generation cephalosporin, imipenem and carbapenem resistance genes and these are important aspects for the future and should be included in the hazard identification part of the expert consultation.

The primary methods of isolation of bacteria have an important and very significant influence on the susceptibility results obtained in monitoring programmes and the methods of primary isolation that are chosen should reflect the study objectives. It is common in surveillance programmes to pick a single colony of the organism in question from primary culture plates containing no antimicrobials (or which contain antimicrobials to which the organism is intrinsically resistant and which are present to reduce contaminants). This isolate is then subjected to susceptibility testing and this procedure gives the likelihood of a bacterium of this species selected at random from the cultured bacterial population being resistant. The alternative approach, involves culturing the primary sample in a selective enrichment stage containing the antimicrobial in question. This latter procedure selects only resistant organisms and detects presence or absence of the resistant organism in the primary sample. This has been important in detection of vancomycin-resistant enterococci (VRE) in samples, where picking a random colony can reveal low or no resistance because VRE are present at low levels; they may still be detectable by selective enrichment.

The suitability of random/ stratified sampling will be partly determined by the prevalence of the target organism. Because of the low prevalence of some veterinary pathogens, collecting these isolates by using stratified random sampling techniques would be costly and would have to be extensive to yield statistically significant numbers of organisms that could be tested to determine emergent resistance at say the 5% level. In the UK, use of clinical veterinary submissions provides useful data for many veterinary pathogens, including *Salmonella* and here, *Salmonella* isolates must also be submitted under statute for susceptibility testing and serotyping if isolated from food producing animals. However for most farmed animals the bulk of samples are in fact recovered from clinical material with the exception of isolates from poultry.

Susceptibility testing programmes have different arrangements to avoid skewing of the results because of differences in the intensity of sampling. For example in DANMAP, a single isolate of each serotype per year from each farm premises is included, whereas in the monitoring system in place in England and Wales, a single isolate of the same serotype over each three month period is included from the same premises. This sampling frequency may be important if resistance is emerging on farm because of ongoing veterinary clinical usage of antimicrobials on premises.

Surveillance programmes should generate data that is suitable to feed into risk assessments. Risk assessors commonly need to know the proportion of the animals that are carrying resistant bacteria and also within an animal host the total numbers of susceptible and resistant organisms to a particular antimicrobial. Existing surveillance programmes may not collect all of the data required for risk assessment.

The WHO Global Principles recommend that surveillance programmes should be designed to be able to determine changes in this rate of emergence of resistance, as well as relate it to overuse, misuse or appropriate use of antimicrobials.

The use of quantitative methodology is preferable in susceptibility test procedures, but whilst countries are moving to either quantitative disc diffusion or MIC-based methods, useful qualitative data may be obtained in surveillance programmes to show trends in development or emergence of resistance. In the absence of suitable quantitative data, available qualitative data should be collected and analysed.

It is unlikely that consensus on clinical breakpoint values for antimicrobials will ever be achieved because of different therapeutic approaches in different countries. The European Society for Clinical Microbiology and Infectious Diseases (ESCMID) has appointed a committee (EUCAST) that are developing an approach in which the susceptibility of the wild-type bacterial population is defined by using quantitative susceptibility data and then deviations from this wild-type population are examined. This approach will allow microbiological breakpoints to be determined.

Resistance data should be interpreted not only by looking at the consumption of antimicrobials in livestock, but also by examining factors which may co-select for antimicrobial resistance (e.g. usage of copper as a growth promoter in the feed of pigs). Over-arching combined summary reports that include consumption data, resistance data and comment on usage of compounds that may be responsible for co-selection should be produced.

The commensals that are normally considered as indicator organisms include enterococci and *E.coli*. It may be appropriate to consider other bacteria as indicator organisms in some circumstances, particularly where one or other of these indicator organisms may be uncommon. Thus, *Streptococcus bovis* could be used in older cattle, *Lactobacilli* in milk or milk products.

G. Conclusion

There is still considerable progress to be made before susceptibility test procedures and surveillance systems for detection of antimicrobial resistance are harmonised at the global level. In the interim, the advice of Arbao I to report quantitative data circumvents some of the pitfalls arising from the use of different breakpoints around the world to differentiate between resistant and susceptible bacterial strains. Adoption of common control strains would also help to harmonise procedures and assist in assessing the overall global situation. Ring trials, such as that conducted in the Arbao II network are also likely to be of immediate benefit and help to overcome the inertia of laboratories to alter their established methods.

Countries should be encouraged to publish their available susceptibility data to allow better assessment of the global situation, identify global data gaps and stimulate further interest in surveillance in this area. There is a need for overall co-ordination of the various attempts that are being made to harmonise and

standardise susceptibility testing at the global level and the OIE Guidelines will provide an important stimulus to help achieve this. There is also a requirement for an ongoing global review to be undertaken of the available surveillance data and its global significance.

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Table 1. Examples of animal bacterial pathogens that may be included in resistance surveillance and monitoring

Target animals	Respiratory pathogens	Enteric pathogens	Udder pathogens	Other pathogens
Cattle	<i>Pasteurella</i> spp.	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	
	<i>Haemophilus somnus</i>	<i>Salmonella</i> spp.	<i>Streptococcus</i> spp.	
Pigs	<i>Actinobacillus pleuropneumoniae</i>	<i>Escherichia coli</i>		<i>Streptococcus suis</i>
		<i>Brachyspira</i>		
		<i>Samonella</i> spp.		
Poultry				<i>Escherichia coli</i>
Fish				<i>Vibrio</i> spp.
				<i>Aeromonas</i> spp.

Table 2. Antimicrobials that may be included in antimicrobial resistance surveillance and monitoring programmes:

Antimicrobial	<i>Salmonella/Escherichia coli</i>	<i>Campylobacter</i>	<i>Enterococcus</i>	Animal pathogens, Gram-positive	Animal pathogens, Gram-negative
Beta-lactams					
Penicillin G				+	
Ampicillin	+	+	+		+
Oxacillin				+ (Staph.)	
Amoxi/Clav	+				
Cephalosporins					
Ceftiofur					
Ceftriaxone	+				
Cephalothin	+				
Macrolides					
Erythromycin		+	+	+	
Lincosamides					
Clindamycin				+	
Streptogramins					
Virginiamycin			+		
Quinupristin/Dalfopristin			+		
Tetracyclines					
Tetracycline	+	+	+	+	
Aminoglycosides					
Streptomycin	+				
Neomycin	+		+	+	
Kanamycin	+		+	+	
Gentamicin	+		+		
Apramycin	+				
Amikacin	+				
Amphenicols					
Chloramphenicol	+				
Florfenicol				+	
Potentiated sulphonamides					
Trimethoprim/Tmp-Sul	+				
\Sulphonamides					
Quinolones					
Nalidixic acid	+	+			
Enrofloxacin/Ciprofloxacin	+	+	(+)	+	+
Glycopeptides					
Vancomycin			+	+	

(+) optional

Chapter 3: Surveillance of Resistant Human Pathogens

Antibacterial resistance in human pathogens has been identified as a major health problem for the past 25 years worldwide. The global rise of this phenomenon presents a growing challenge to medicine and public health. This problem has been addressed in several WHO documents.

Surveillance systems were developed in all developed countries and in many developing countries. Coordination and networking of numerous systems were established on both national and international levels (Ref. D. Monnet, CDC program, WHO net program).

Nosocomial infections organisms were the most often surveyed. Community-acquired infections and food-borne infections are less properly reported in many countries despite their incidence and the WHO recommendations.

The most challenging pathogens causing human nosocomial infections are:

- *Methicillin resistant staphylococci*
- *Acinetobacter baumannii*
- *Pseudomonas aeruginosa*
- *Enterobacter cloacae*
- *Enterococci*

While in community-acquired infections and foodborne diseases:

- *Strep pneumoniae*
- *Strep pyogenes*
- *Neisseria gonorrhoeae*
- *Mycobacterium tuberculosis*
- *E. coli (special serotypes)*
- *Salmonellas*
- *Campylobacters*

The nonhuman use of antibiotics has clearly contributed to the development of multi-resistance in *E. coli*, *salmonella*, *campylobacter* and *enterococci*.

It is worth-mentioning that the following food-borne pathogens: *Listeria*, *Yersinia*, *Vibrio* and *Brucella* have not developed resistance to antimicrobials till now.

Surveillance systems for human pathogens must be integrated to surveillance systems for zoonotic bacteria in animals. This task has been achieved in USA, UK, Denmark, Holland, Finland, Sweden, Norway, Germany and France. It offered new tools for research:

- Comparative epidemiology
- Molecular analysis of different phenotypes and outbreaks
- Specific molecular investigation on resistance (mechanism, prevalence, trends...)

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Chapter 4: Factors contributing to emergence and spread of resistance in the food production chain

A. Summary

Antimicrobial resistance emerges in primary food production in response to antimicrobial selective pressure. Movement of animals, animal manure, and food- and by products facilitates spread of resistance. Bacterial factors, such as fitness of the clone to the environment, as well as resistance to antimicrobials, can promote the spread of some clones over others in the food production chain. Some resistant bacteria emerged in agriculture can cause human infections, whereas others, can pass their resistance determinants, by mode of horizontal transmission, to human pathogenic bacteria in humans. Resistance can spread from agriculture to humans by a multitude of routes. The foodborne route is probably the most prominent.

B. Emergence of resistance

Resistance is classified as either intrinsic or acquired. Bacteria can be intrinsically resistant or naturally resistant to antimicrobials e.g. if they lack the cellular mechanism required for the antimicrobials action. Acquired resistance occur due to chromosomal mutation or through acquisition of transferable genetic material.

Mutational resistance

Mutations result from rare mistakes in the DNA replication process. Most chromosomal resistance mutations results in alteration in the permeability of specific antibiotic target sites. These mutations are divided into single-step and multi-step types and this resistance is vertically spread. For example, resistance to quinolones is mediated by a mutation in the chromosomal gene encoding for the production of an enzyme, which assists in the DNA transcription process (an RNA gyrase). In *Enterobacteriaceae* a single mutation results in “low-level resistance” to quinolones (as determined *in vitro*) whereas it requires a two-step mutation for “high-level resistance” to emerge. In contrast, in *Campylobacters* a single mutation is sufficient to develop high-level resistance to quinolones. Resistance emerging through mutation develops *in vitro*, for instance in a classical culture flask experiment, when a pure culture of the bacterium is exposed to increasing concentrations of the drug. It only requires the bacterium and the drug; there is no need for foreign DNA from resistant bacteria. Consequently, resistance emerges spontaneously during treatment of animals and humans, and it spreads vertically by spread of the resistant bacterial clone (Acar et al., 1993).

Mutational resistance emerges as random events. Exposure to the drug provides an environment that favours the propagation of the mutant over the wild-type, and consequently increases the probability that the resistant clone will spread to a neighbouring niche.

Acquired resistance

Before patients were first treated with antimicrobial agents, only 65 years ago, bacteria isolated from them had almost no resistance genes. However, after each new agent became widely used, a gene expressing resistance to it ultimately emerged. Emergence means here that the resistance gene,

wherever its origin, had spread enough to get into a strain of a species that was isolated and noticed as resistant by a clinical laboratory somewhere (Hughes & Datta, 1983).

We can infer from DNA homology what the remote ancestors of some resistance genes may have been (e.g. the strain of filamentous fungus that produces the antimicrobial). However, we know little of the events that occurred between an agent became widely used and the time the gene expressing resistance to it emerged in a clinically important bacteria. After each antimicrobial agent had become widely used, it presumably encountered a strain of bacteria somewhere that expressed at least some slight level of resistance to the agent. Antimicrobial agents are dosed to attain high concentrations at sites of tissue infection, but gradients down to trace levels in nearby niches can give advantages to strains just resistant enough to survive such trace levels. Furthermore, some uses, like the use for growth promotion, actively seek to attain trace levels of drugs for extended periods of time in the exposed animal. Resistance may evolve in any exposed species, and the evolved resistance gene might transfer on genetic vectors, perhaps repeatedly, before reaching a species that would ultimately be isolated in a clinical laboratory. Whatever the molecular details of the emergence of each resistance gene, and wherever they went on, the time elapsed and the amounts of agent use before emergence have usually been great. This suggests that an enormous number of encounters between agent and germs are needed to produce the first emergence of most resistance genes. Eventually many different genes have emerged to express resistance to some agents, such as tetracyclines and macrolides, while only three have been described to express resistance to the oldest agents, sulfonamides. Some resistance genes may emerge repeatedly from different origins, while others appear to have spread widely from a few emergences – or perhaps only one (O'Brien, 2001).

Resistance genes are most often encoded in extrachromosomal genetic elements or in segments that appear to have been recombined into the chromosome from other genomes. The largest of the extrachromosomal elements are the plasmids, which are self-replicating, double-stranded circles of DNA, some of which express mechanisms that transfer the plasmid to another bacterial cell. Bacteria isolated from patients 70 years ago or more, before antimicrobials were first used, had plasmids similar to those seen now, but then, the plasmids had no resistance genes (Datta & Hughes, 1983; Hughes & Datta, 1983).

Resistance genes encoded in plasmids are often located within segments called transposons. Functioning transposons include transposases that enable the transposon to recombine into other genomes. Such recombination can be demonstrated *in vitro*; evidence *in vivo* is provided by transposons with identical nucleotide sequences on a variety of different plasmids (Pembroke et al., 2002).

Resistance genes are often further clustered within elements called integrons, which are frequently found within transposons and plasmids but also found in bacterial chromosomes. Each resistance gene in an integron is encoded in a mobile gene cassette that can be excised and then incorporated into another integron on another genome. Multiple cassettes with different resistance genes are commonly lined up, one after another, in an integron and expressed as a group from one upstream promoter (Hall & Collis, 1995). The sequential order of genes often reflects the usefulness of each resistance factor to the bacterium in its environment, with the genes most commonly needed being close to the promoter and those less often required being further away. This means that the genes most commonly needed are most transcribed, because transcription often stops before all of the genes in the sequence have been read. This is thought to play a role in ensuring that bacterial fitness is not compromised by carriage of additional resistance genes, since only the genes that are most needed are

transcribed at higher levels. Successful bacterial clones may be particularly adapted to their environment in this way (O'Brien, 2001).

The wealth of gene recombination and transfer mechanisms might predict a near-infinite diversity of genetic resistance elements in clinical isolates. Whenever looked for in molecular detail, however, as seen in examples presented later, certain genetic elements, constructs, plasmids, and bacterial clones predominate in parts of the world or even throughout the whole world. Such observations remind us that resistant bacteria compete not only with susceptible bacteria but also with one another, both in the presence and in the absence of antimicrobial agents. These considerations suggest the need for ongoing intricate evolutionary adjustments for competitive success.

If a resistance gene has emerged on the chromosome of a bacterial strain, its spread may depend mostly on that strain and thus be restricted by the fitness of that particular strain for various niches. However, the mechanisms described above can mobilize the resistance gene into another strain or species (e.g., genes for tetracycline resistance recombined from *E. coli* into *Salmonella*) or into a mobile genetic element.

A resistance gene that has emerged on a plasmid or become inserted into one later may be transferred to other strains and species fit for niches not accessible to its original host strain. The resistance gene might also be within a transposon, an integron, or both, which could mobilize it to a different plasmid able to transfer to additional strains and species. Insertion of a resistance gene into progressively more plasmids carried by more strains and species extends its range and enables it to penetrate into more niches and persist longer after each antimicrobial exposure. Such insertions may put the resistance gene on plasmids already carrying genes expressing resistance to other agents, or even into integrons sharing promoters with such genes. Any of those agents will thereafter select for all of these now-linked resistance genes (co-selection).

A rare mutational or recombinational event is required for each step in the evolution of genetic vectors encoding resistance genes. The chance of each such event occurring depends on the prevalence of the construct produced by the preceding event. This multi-step assembly of increasingly fit, antimicrobial resistant genetic constructs would thus be expected to occur mostly where large populations of bacteria are kept under intense antimicrobial selection to amplify and concentrate the elements being assembled. Such places may serve as unintended recombinant DNA "hot spots" for the development and export of more competitive resistance vectors. The food animal GI tract represents such a "hot spot" (O'Brien, 2001). This is well-illustrated by considering the bovine mastitis streptococci. *Streptococcus agalactiae*, which is restricted to the udder as an obligate udder pathogen only very rarely shows resistance, whereas *Streptococcus uberis* which frequently inhabits the bovine intestine and bovine faeces tends to be resistant to a much wider range of antimicrobials. The host associated *Salmonella* serotypes Enteritidis and Dublin, that primarily colonize the reproductive organs of the preferred host animal likewise have lower levels of resistance than the serotypes with a wide host range and primarily colonizing the GI tract of food animals e.g. Typhimurium, Infantis, and many others. The potential for exchange of resistance determinants in the intestine may, at least in part, account for these differences.

C. Selection of resistance

The basic event in selection is simple. Enough molecules of the antimicrobial agent impinge on a bacterial cell that is about to divide to stop it from doing so, while in its place another cell divides that

would not otherwise have divided. The second cell divides either because it was not inhibited by the same exposure (i.e., had some level of resistance) or because it did not quite get that same exposure (e.g., by being a bit away and coming into the space later). We may try to estimate how many times that basic event occurs at any place as a measure of the magnitude of antimicrobial selection there. This measure would reflect both the size of the bacterial populations being exposed to antimicrobials and the duration of that exposure. Such a measure might, for example, show the magnitude of selection in the intestines and the environment of all of the cattle, pigs, and poultry in a country to equal or exceed that in humans, even if the tonnage of antimicrobials given to animals were less than that given to humans (O'Brien, 2001).

Beyond this basic event, other variables may supervene. Exposure to low concentrations of antimicrobials may select for some types of resistance that progress by small increments and would be obliterated by higher concentrations. Genetic vectors of resistance that were concentrated in certain bacterial populations by prolonged exposure to antimicrobial agents might have a greater chance to recombine and evolve to greater efficiency than those allowed to become sparse during intervals of no exposure. Certain agents may drive the evolution and spread of resistance vector constructs more effectively in certain populations of bacteria because of the previous deployment of particular resistance genes and vectors (Doucet-Populaire, 1991).

In the context of the food production chain selection of antimicrobial resistance takes place in all niches where the bacterial cells may divide in the presence of antimicrobial agents. From a bacterial population perspective this is primarily the GI tract of animals exposed to antimicrobials for therapy, prophylaxis or growth promotion. It may also, to a lesser degree, be in the farm or food production environment where exposure to detergents, acids or other agents with antimicrobial properties may provide a favourable selective pressure. Animal and human wastes may contain traces of antimicrobials as a result of dilution or natural breakdown of the antimicrobial product and these levels may still be high enough to exert a selective effect in some cases. It is furthermore, important to recognize that the fitness of a bacterial clone depends on many factors, such as resistance to adverse environmental conditions (heat, pH, water activity, etc.), which, in addition to antimicrobial resistance, will influence, and ultimately determine, the success of the clone in the agricultural- and food processing environment.

D. Spread of resistance

To fully appreciate the power of the food production chain to spread resistance, one has to recognize both the potential for bacterial spread, irrespective of antimicrobial resistance, as well as the additional impact that antimicrobial selection has on the spread of antimicrobial resistant strains. Within food animal populations, massive international spread of human pathogenic bacterial clones has occurred, for example *Salmonella* Enteritidis phage type 4 in the broiler and shell egg production chain. The international spread takes place primarily with the trade of pedigree animals for breeding, and national spread occurs subsequently through the national breeding pyramid. Vertical transmission through increasingly limited numbers of internationally integrated breeding pyramids of food animals, constitutes a powerful system for spread of human pathogenic bacteria, that are generally benign to the animals and consequently not controlled by animal health restriction in relation to animals in international trade. Most enteric bacteria, such as *Salmonella*, *E.coli*, *Yersinia*, and *Campylobacter*, have the potential to spread vertically, by faecal oral transmission at birth, between

progeny and offspring, and consequently by the mechanism described above (Ranta & Maijala, 2002, Thorns, 2000).

Animal manure is spread in the environment, mostly without any decontamination steps. Consequently, active unmetabolized antimicrobials as well as resistant bacteria are spread in the immediate environment of the farm (Sengeløv et al., 2003). Resistant bacteria deposited in the environment can spread to other food animals, wild animals, and/or to humans. Furthermore, if manure is deposited on farmland resistant bacteria from animals can transmit to humans with food plants (Witte, 2000). Resistant bacteria and unmetabolized antimicrobials deposited in the environment can also spread to the ground water reservoirs (Halling-Sørensen et al., 1998; Chee-Sanford et al., 2001)

During slaughter of food animals faecal contamination of the carcass occurs. The degree of contamination may vary with the level of hygiene, however some level of contamination always occurs. Furthermore cross-contamination can take place between contaminated and un-contaminated foodstuffs at any stage of the food production chain as well as in the consumers kitchen.

The final critical step is the oral intake of sufficient numbers of bacteria to successfully pass the gastric barrier and enter the intestinal tract of humans. Such intake can occur by ingestion of contaminated food (either cross-contaminated non-heated, or insufficiently heated), by hand mouth activities including thumb sucking in children, by contact with faecal material from pets exposed to raw meat, etc. There are multitudes of routes, as documented during detailed investigations of foodborne disease outbreaks.

Transmission between humans can also take place, both in the household, or in settings where such transmission is facilitated by crowding such as day-care centers and hospitals.

In recent years, quantitative risk assessments of the spread of *Campylobacter*, *Salmonella* and *E. coli* in the farm-to-fork chain have evaluated the spread of bacteria from animals to humans. The effect on this transmission chain exerted by antibiotics is increasing the numbers of resistant bacteria in the animal reservoir (relative to sensitive strains), and thus, the number of resistant bacteria that contaminate the food during primary processing in the abattoir. Furthermore antimicrobials in humans may increase the patients' susceptibility to infections by the resistant strains as shown in a number of studies. So the effect of antimicrobial resistance is both one of propagation in the primary end of the chain and one of predisposition in the final stage of the transmission chain.

Thus, a number of factors may potentially be considered to influence the spread of successful bacterial clones in animal and human populations, including:

- The environmental “fitness” of the bacterial organism – its ability to survive and compete with other bacteria in the same and other niches.
- The potential to exploit new environments, in addition to those inhabited by “wild-type” strains.
- Resistance to adverse environmental consequences, which might range from physical factors, such as drying, to chemical factors such as ability to withstand low pH or disinfectants.
- The ability to colonise animal and human hosts and the degree of multiplication that can occur within these hosts.
- The ability to withstand host immunity.

- The structure of the livestock industry. Infection at the upper stages of the pyramid in elite breeding animals will lead to dissemination down the chain to production stock.
- Animal husbandry procedures, such as disposal of slurry and movement of animals (particularly mixing at sale of a number of animals and then dispersal to a number of different farms)
- Resistance to antimicrobials used in treatment or for other purposes.

Examples of emerged resistance constructs spreading throughout the world.

The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades. This increase has been judged to be primarily the result of selection pressure exerted by antimicrobials in the feeds of food-producing animals. However, clonal dissemination also plays an important role for both human and animal infections with *Salmonella*, and this is not driven only by antimicrobial selection pressure but also by movement of animals and food products.

Multidrug resistant *Salmonella* Typhimurium

The increase of a penta-resistant subtype of Typhimurium was observed in the United Kingdom, Europe, and North America in the 1990s. This subtype was primarily identified by two phenotypic markers: 1) resistance to at least the five antimicrobials (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline [ACSSuT]) and 2) the definitive phage type 104 (DT104). Molecular genetic studies conducted in Great Britain, France, Ireland, Germany, Denmark, and the United States²⁰ have contributed to the consensus that mr-DT104 represents a globally disseminated clone. Mr-DT104 emerged from an unknown location and has spread globally very rapidly, as it was first detected in Europe, Asia, and North America¹⁰ almost simultaneously. The earliest isolations of mr-DT104 were from humans and birds in the United Kingdom in 1984, and from a human in the United States in 1984 and 1985. The first isolates from agricultural animals were observed in Britain in 1988 and in the United States in 1990. The species and location in which the mr-DT104 clone originated are unknown (Davis et al., 2002; Cloeckaert & Schwarz, 2001; Threlfall et al., 2000; Hollinger et al., 1998).

The rapid international dissemination of mr-DT104 is consistent with human travel and international trade of breeding animals. Travel is a well-established risk factor for salmonellosis in developed countries, and top breeding animals in international trade have been shown to carry DT104. The relative impact of each mode of transmission is unknown. In the case of mr-DT104, the clone has successfully replaced other resistant *Salmonella* Typhimurium clones in some settings, suggesting that additional fitness advantages have allowed mr-DT104 to disseminate. These factors have not yet been identified.

In addition to mr-DT104, other phage types of the serovar Typhimurium have demonstrated an ability to disseminate widely, producing marked changes in the prevalence of antimicrobial resistance in a region. In the 1960s, a multiresistant Typhimurium phage type 29 was disseminated in the United Kingdom by means of the sale and distribution of infected calves, and became prevalent among dairy cattle and humans until 1969. In the 1970s, phage types 204, 193, and 204c rose to prominence among cattle-origin *Salmonella* Typhimurium isolates.⁶ Type 193 was derived from CSSuTresistant Type 204 by the acquisition of a plasmid that encoded additional resistance to ampicillin and kanamycin. Type 204c differed from Type 204 by an additional resistance to trimethoprim (and thus it was CSSuTTm). Phage types 204 and 193 also became disseminated internationally by calf traders who sold infected calves to locations throughout the United Kingdom and in Europe. Although

multiresistant phage type 204c was prevalent among cattle in the UK through the beginning of the 1980s, the proportion of human Typhimuriums that were phage type 204c remained low. The epidemic in calves of DT204c peaked in 1986 and was ending in 1993 while mr-DT104 was on the rise. Clones of non-Typhimurium *Salmonella* serovars have also disseminated regionally and internationally. A human-adapted serovar with multiple resistance, *Salmonella* Wien, disseminated through Europe from Northern Africa. After having first been reported in association with an Algerian pediatric ward in 1969, Wien became the most frequently isolated serovar in France and Italy in the 1970s. Disseminated multi-resistant clones of *Salmonella enterica*, after a period of increase in proportion of total isolates, typically decline to become relatively minor subtypes (Davis et al., 2002). The mechanism of expansion and subsequent replacement of *Salmonella* clones in both human and animal populations is unknown.

Vancomycin-resistant enterococci

In enterococci there are four different genotypes of acquired glycopeptide resistance of which the VanA genotype is the most frequent in Central Europe. The mechanism of glycopeptide resistance mediated by the VanA gene cluster is based on target alteration (formation of a depsipeptide instead of the D-Ala-D-Ala group of N-acetylmuramic acid). The gene cluster is located on transposons of the Tn 1546 type, which are integrated into conjugative plasmids. The detection of glycopeptide-resistant *Enterococcus faecium* (GREF) possessing VanA in wastewater treatment plants of small towns with no hospitals in Germany was the first indication of a reservoir outside hospitals (Witte, 2001). The assumption that the use of the glycopeptide avoparcin as feed additive created a reservoir of GREF in animal husbandry was confirmed by the demonstration of GREF in animal feces (pigs and chickens) from farms using avoparcin but not, or only rarely, in those not using avoparcin (Bager et al., 1999). The presence of GREF in the intestinal flora of meat animals also suggested their presence in meat products, which was demonstrated for poultry carcasses and raw minced pork. If GREF was in meat products, a spread to healthy, non-hospitalized humans became likely, and has been confirmed (Stobberingh et al., 1999; Wegener et al., 1999; Witte, 2001; van den Bogaard et al., 2002). These data demonstrated the route of dissemination from meat animals to humans in general. In order to trace the spread of transferable resistance the following questions had to be answered: 1) Is there a clonal spread of particular GREF strains among animals and humans? 2) Does glycopeptide resistance spread by transfer of the VanA gene cluster among different *E. faecium* strains? 3) Is the structural configuration of the VanA gene cluster uniform in GREF from human and animal sources or are there different “types” in enterococcal populations of humans and of meat animals? Data available to date have led to the following conclusions: a variety of different SmaI-macrorestriction as well as multilocus electrophoresis patterns demonstrate the polyclonal nature of GREF and suggest a frequent dissemination of the VanA gene cluster among different strains. When a GREF strain of porcine origin was ingested by a human volunteer, it persisted over several months (Sorensen et al., 2001). Plasmids from GREF of different ecological origin exhibit rather different restriction endonuclease cleavage patterns; this suggests a frequent transposition of the VanA gene cluster among different plasmids. Different structural types of the VanA gene cluster resulting from integration of insertion sequences into non-coding regions or from deletions concerning genes for transposition have been described (Woodford, 2001). If the animal and the human reservoir of GREF communicate, the same structural types should be demonstrated. Until now different molecular methods have been applied to molecular typing of the VanA gene cluster in different countries. The AFLP typing method (the most discriminatory typing methods for enterococci) has shown that human faecal isolates from hospitalized humans belong to clusters shared by animal isolates, supporting animal-to-human spread (Bruinsma et al., 2002). Besides a wide spread of Tn1546 like elements in

their original configuration, there are different types that are characteristic for GREF from chicken and pigs. In humans, however, “animal specific” types of the VanA gene cluster have been found, which furthermore demonstrates dissemination from the animal reservoir (Jensen et al., 1998).

Resistance in *E. coli*

For every *Salmonella* in the colons of humans or animals, there are thousands or more *E. coli*, subject to the same antimicrobial selection and capable of carrying and spreading the same or similar genetic resistance elements. The abundance of *E. coli* implicates them as the likely predominant vehicles for the spread of resistance genes and vectors, as opposed to the spread of infection, between the bacterial populations of animals and humans; however, their abundance also makes such spread difficult to trace. A new strain is unlikely to be noticed, especially if the strain does not cause illness, and there is little typing of *E. coli* strains. However, there is a screening and serotyping program to detect *E. coli* strain O157:H7, a pathogenic strain, and there are now many examples of this strain spreading from animals to humans. If antimicrobial-resistant *E. coli* from animals also flows to humans in similar proportion, they would be the major route for such spread of resistance. Pioneering work was performed by Levy and co-workers on dissemination of plasmid encoded oxytetracycline resistance in *E. coli* demonstrating spread from farm animals to humans working and living on farms (Levy, 1978). At that time however, oxytetracycline resistance was already very frequent in coliforms in humans as well as other animals, and there was also a substantial use in human chemotherapy. Once a resistance gene has already become widely disseminated among different ecosystems, it is always difficult to trace it back to its origin. This is only possible by genetic labeling of a particular resistance gene and following its further spread or by prospective studies after the introduction of an antibiotic for use in only one field of application such as animal feeding. The latter became possible after the use of oxytetracycline as growth promoter was stopped and replaced by a streptothricin antibiotic in the former East Germany in 1983. No resistance was seen in *Enterobacteriaceae* from animals and humans at that time. The first occurrence of a transposon-coded resistance mechanism (streptothricin acetyltransferase) was observed 2 years later in *E. coli* from the gut flora of pigs. By the time its use was stopped after the German reunification in 1990, resistance had spread to *E. coli* from the gut flora of pig farmers, further family members and also to *E. coli* from citizens in municipal communities and from urinary tract infections. The resistance determinants obviously spread in the absence of any selective pressure. Finally, the resistance determinant was detected in *Salmonella* and *Shigella* spp. isolated from cases of diarrhoea. Two recent lines of evidence further indicate a role for *E. coli* in the spread of resistance between animals and humans (Witte, 2001). Studies from Spain and Taiwan, where quinolones are used in commercial poultry production, have found that a large proportion of retail chicken carcasses now carry strains of *E. coli* with reduced susceptibility to quinolones; a rising percentage of isolates from humans, including children, also have reduced susceptibility. Because quinolones are not indicated for treatment of children, the resistance presumably did not originate within the *E. coli* bacteria while the children were carrying them (McDonald et al., 2001). Another recent report found the same multidrug-resistant clone of *E. coli* to be causing urinary tract infections in multiple patients at widely separated locations in 3 different states in the United States; a similar report from the United Kingdom a decade ago was referenced (Manges et al., 2001). Neither directly identified a food source. The authors of the report did, however, question how a clone could gain such wide distribution and noted the similarity of that distribution to that of multistate *Salmonella* outbreaks due to a contaminated food product distributed through the food chain.

Plasmid-mediated spread of resistance

Salmonella Newport has recently emerged in North America to become the third most common serotype causing salmonellosis in man for the past few years. Spread and disease have also occurred in animals, mainly in cattle. The organism commonly shows pentaresistance to five antimicrobials in the ACSSuT pattern and some strains additionally show resistance to third generation cephalosporins, via the possession of *cmv2*, a beta-lactamase gene that is normally present on the chromosome of *Citrobacter freundii*, but which has “escaped” onto a plasmid in multi-resistant *Salmonella* Newport strains. This plasmid now appears to be spreading to other serotypes of *Salmonella* and has been detected in Agona and Heidelberg in North America. Further evidence of spread has been shown by the detection of *cmv2* in an *E.coli* O157 isolate. *Citrobacter freundii* is commonly found in many countries and wild-type isolates naturally carry *cmv2* on their chromosome. However, *cmv2* in a transferable plasmid vector obviously has very different properties from *cmv2* stably-integrated within a host chromosome and the available evidence is starting to suggest it now has the potential to spread to a range of other enterobacteriaceae (Rankin et al, 2002; Zansky et al., 2002; Allen & Poppe, 2002).

E. Review of previous WHO/FAO/OIE consultations and reports

The findings in this chapter, which is largely based on recent scientific reviews and new research findings, support and further substantiate the below conclusions from the 1997 WHO expert consultation.

From: The Medical Impact of the Use of Antimicrobials in Food Animals. Report of a WHO Meeting, Berlin, Germany, 13-17 October 1997

Antimicrobial use leads to the selection of resistant forms of bacteria in the ecosystem of use. This will occur with all uses including treatment, prophylaxis and growth promotion. Examples of factors influencing the development of resistance include drug concentration, long-term exposure, organism type, antimicrobial type and host immune status. Low-level, long-term exposure to antimicrobials may have a greater selective potential than short-term, full-dose therapeutic use. Resistance can be selected in both target bacteria and other exposed bacteria, with resulting adverse consequences for the prevention and treatment of diseases in humans, animals and plants.

Bacteria and genes, including resistance genes, can pass between human, animal and other ecosystems. When resistant bacteria are themselves pathogenic or can transfer their resistance genes to pathogenic bacteria, adverse health effects can result.

Antimicrobials are used in animals as growth promoters (in subtherapeutic doses), prophylactically for disease prevention (for example, after commingling of animals from different farms) or therapeutically, for treatment of infections. Adverse consequences of selecting resistant bacteria in animals include:

- an increase in the prevalence of resistant bacteria in animals; the transfer of resistant pathogens to humans via direct contact with animals, or through the consumption of contaminated food or water;
- the transfer of resistance genes to human bacteria;
- an increase in the incidence of human infections caused by resistant pathogens; and
- potential therapeutic failures in animals and humans.

Residues of antimicrobial agents in food of animal origin in excess of the agreed acceptable minimum residue levels (MRLs) may contribute to the generation of resistance in bacteria in humans. However, the current evidence suggests that the risk is low. Of more concern may be that such residues could indicate inappropriate use of antimicrobials by the producer.

The medical consequences of resistance acquisition in bacteria of animal origin are highlighted by the following examples.

Salmonella

There is direct evidence that antimicrobial use in animals selects for antimicrobial-resistant nontyphoid *Salmonella* serotypes. These bacteria have been transmitted to humans in food or through direct contact with animals. Antimicrobial resistance limits the therapeutic options available to veterinarians and physicians for the subset of clinical cases of nontyphoid *Salmonella* which require treatment. A recent example is a clone of *S. typhimurium* DT104, resistant to ampicillin, tetracycline, streptomycin, chloramphenicol and sulphonamides, which has become prevalent in many countries including the United Kingdom, Germany and the United States of America.

Following the introduction of fluoroquinolones for use in food-producing animals, the emergence of *Salmonella* serotypes with reduced susceptibility to fluoroquinolones in humans has become a cause for particular concern. This phenomenon has been observed in countries such as France, Germany, Ireland, the Netherlands, Russia Federation, Spain and the United Kingdom.

Campylobacter

Following the introduction of fluoroquinolones for use in poultry there has been a dramatic rise in the prevalence of fluoroquinolone-resistant *Campylobacter jejuni* isolated in live poultry, poultry meat and from infected humans. Moreover, prior to any use in poultry, no resistant strains were reported in individuals with no previous exposure to quinolones. Fluoroquinolone-resistant *C. jejuni* has been associated with therapeutic failures in humans.

Enterococci

The use of avoparcin as a growth-promoting feed additive in animal husbandry has contributed to the reservoir of transferable resistance genes to glycopeptides, including vancomycin, in the commensal enterococci of animals. Glycopeptide-resistant enterococci from animals can reach humans via the food chain. Although glycopeptide resistance genes have been shown to be widely disseminated, the extent to which the gene pool in animals contributes to the prevalence of glycopeptide-resistant commensal enterococci in humans has not been quantified. Glycopeptide-resistant enterococci cause serious infections in hospitalised immune-impaired patients. In this setting they contribute to increased morbidity and mortality, in part because of limited therapeutic options. This medical impact would be greatest in countries where vancomycin is used intensively.

There is concern that there will be increased dissemination of glycopeptide resistance genes to *Enterococcus faecalis* and their spread to other gram-positive organisms, particularly to multiresistant *Staphylococcus aureus* for which vancomycin is the drug of last resort. Due to the limited number of agents available for the treatment of glycopeptide-resistant enterococci, antimicrobial agents not previously used in humans are being sought, including drugs from classes currently used as growth

promoters in animals. Therefore the selection of further resistance in enterococci is undesirable, e.g., streptogramin resistance due to use of virginiamycin as a feed additive in animals.

Escherichia coli

Multiresistant *Escherichia coli* have been selected by the use of broad-spectrum antimicrobials in both livestock and humans. The development of antimicrobial resistance in *E. coli* creates problems due to their high propensity to disseminate antimicrobial resistance genes. Resistance genes have been traced from *E. coli* in animals to *E. coli* in humans. Certain *E. coli* are foodborne pathogens and most of these strains are currently susceptible to antimicrobials. Should therapy be required, it could be compromised by the development of resistance in these strains.

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Chapter 5: Evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans, and the human health consequences of such resistance

As in human medicine, the use of antimicrobial agents in food animals creates a selective pressure for the emergence and dissemination of antimicrobial-resistant bacteria. Antimicrobial resistance resulting from the use of antimicrobial agents in food animals may occur among animal pathogens, commensal bacteria that are present in food animals, and human pathogens that have food animal reservoirs. These resistant bacteria may be transferred from food animals to humans by various means including through the food supply and following contact with animal manure. The transfer of resistant bacteria from food animals to humans is most evident in, but not limited to, human pathogens which have food animal sources, such as *Salmonella*, which has important reservoirs in cattle, chickens, pigs and turkeys, and *Campylobacter*, which has reservoirs in chickens and turkeys.

This chapter will describe the conclusions of previous WHO/FAO/OIE consultations and reports, and review (1) evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans, and (2) evidence that such antimicrobial resistance results in human health consequences.

A. Review of previous WHO/FAO/OIE consultations and reports

Association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans

Many expert panels, including WHO Consultations, national committees, and independent organizations, have examined the association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans. WHO organized two consultations, in Berlin in 1997 and in Geneva in 1998, to qualitatively assess the risk of human health consequences associated with the use of antimicrobial agents in food animals. The Berlin meeting was entitled “WHO Consultation on the Medical Impact of Antimicrobial Use in Food Animals.” At this meeting, it was concluded that “there is direct evidence that antimicrobial use in animals selects for antimicrobial-resistant nontyphoid *Salmonella* serotypes. These bacteria have been transmitted to humans in food or through direct contact with animals.”

Because of the human health importance of fluoroquinolones and public health concern of increasing resistance to fluoroquinolones, particularly among *Salmonella* and *Campylobacter*, the WHO Consultation in Geneva focused on the risk of human health consequences associated with the use of fluoroquinolones in food animals. This meeting was entitled “Use of Quinolones in Food Animals and Potential Impact on Human Health.” It was concluded at this meeting that “the use of fluoroquinolones in food animals has led to the emergence of fluoroquinolone-resistant *Campylobacter* and of *Salmonella* with reduced susceptibility to fluoroquinolone.”

Similar conclusions have been presented to two committees of the Codex Alimentarius Commission: the Codex Committee on Food Hygiene (CCFH) and Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). A “Risk profile on antimicrobial-resistant bacteria in food” presented to the thirty-fourth session of CCFH in August, 2001, stated that: “Antimicrobials are used in food animals for growth promotion, prophylaxis, metaphylaxis, and therapy. This use is the principle contributing factor to the emergence and dissemination of antimicrobial resistance among bacterial pathogens and

commensals that have food animal reservoirs.” Similarly, a “Discussion paper on antimicrobial resistance and the use of antimicrobials in animal production” presented to the thirteenth session of the CCRVDF in July, 2001, stated that: “Animals serve as reservoirs for foodborne pathogens, including *Salmonella* and *Campylobacter*. Antibiotic resistant foodborne pathogens may be present in and on animals as a result of drug use in animals. These resistant foodborne pathogens may contaminate a carcass at slaughter and can be transmitted to humans through consumption and handling of contaminated food. In industrialized countries, the foodborne pathogens, *Salmonella* and *Campylobacter*, are infrequently transferred from person to person. In these countries, epidemiological data have demonstrated that a significant source of antibiotic resistant foodborne infections in humans is the acquisition of resistant bacteria originating from animals that is transferred on food.”

Conclusions from these WHO Consultations and presentations to the Codex Alimentarius Commission suggest agreement among scientific experts that use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, particularly antimicrobial-resistant *Salmonella* and *Campylobacter*, that have been transmitted to humans. This suggests sufficient evidence, particularly among *Salmonella* and *Campylobacter*, to demonstrate an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans.

B. Human health consequences resulting from such resistance

In contrast to the sufficient evidence demonstrating an association between antimicrobial use in food animals and antimicrobial resistance among bacteria isolated from humans, little evidence of human health consequences of such resistance was presented at the WHO consultations or in presentations to the Codex Alimentarius Commission. However, these consultations and presentations focused on treatment failures and did not review the full range of potential adverse human health consequences of such resistance. The consultants at the Berlin meeting in 1997 concluded that “microbiological and clinical evidence is mounting that resistant bacteria or resistant determinants might be passed from animals to humans resulting in infections that are more difficult to treat”, but “the magnitude of the medical and public health impact of antimicrobial use in food animal production is not known.” The WHO Consultation in Geneva concluded that “there has been little documented impact of this resistance on human health to date, but there is concern about the potential human health consequences if resistance were to increase and spread. Further research and data gathering are essential to quantify this potential.”

C. Evidence

Association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans

There is clear evidence that the use of antimicrobial agents in food animals is associated with antimicrobial resistance among bacteria isolated from humans. Evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans is most evident among *Salmonella* and *Campylobacter*, but is also present among enterococci, *Escherichia coli*, and other bacteria. The awareness of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans is longstanding. In the United Kingdom, for example, the “Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine,” chaired by Professor Swann, concluded in 1969 that “It is

clear that there has been a dramatic increase over the years in the number of strains of enteric bacteria of animal origin which show resistance to one or more antibiotics. Further, these resistant strains are able to transmit this resistance to other bacteria. This resistance has resulted from the use of antibiotics for growth promotion and other purposes in farm livestock. There is ample and incontrovertible evidence to show that man may commonly ingest enteric bacteria of animal origin.”

Several lines of evidence demonstrate an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans including: (1) outbreak investigations, (2) epidemiological investigations, (3) field studies, (4) case reports, (5) ecological and temporal associations, and (6) molecular subtyping. Numerous studies provide support for one or more of the lines of evidence demonstrating an association between use of antimicrobial agents in food animals and antimicrobial resistance in humans.

Several authors have recently summarized these lines of evidence (Angulo et al, 2000; Swartz, 2002). Although most of these lines of evidence were available at the WHO Consultations in Berlin and Geneva, additional data has accumulated since these consultations to support several of these lines of evidence.

(1) Outbreak investigations – Although outbreaks only represent a fraction of the cases of infections caused by foodborne pathogens, including *Salmonella*, much insight into the epidemiology of foodborne diseases has been provided through investigations of outbreaks. Several outbreak investigations of antimicrobial-resistant *Salmonella* infections in humans have combined epidemiological fieldwork and laboratory subtyping techniques to trace antimicrobial-resistant *Salmonella* through the food distribution system to farms, and use of antimicrobial agents on the farms was found to be associated with the antimicrobial resistance in the *Salmonella* isolated from humans. Among the most notable outbreak investigations have been the tracing of human tetracycline-resistant *Salmonella* infections to the “top-dressing” of cattle feed with tetracycline (Holmberg et al, 1984) and the tracing of human chloramphenicol-resistant *Salmonella* infections to the illegal use of chloramphenicol on dairy farms (Spika et al, 1987). More recently, an outbreak of human nalidixic-acid resistant *Salmonella* Typhimurium DT104 infections in the United Kingdom was traced to a dairy farm where fluoroquinolones were used in the dairy cattle in the month prior to the outbreak (Walker et al, 2000). Furthermore, a review of outbreaks of *Salmonella* infections indicated that outbreaks caused by antimicrobial-resistant *Salmonella* were more likely to have a food animal source than outbreaks caused by antimicrobial-susceptible *Salmonella* (Holmberg et al, 1987)

(2) Epidemiological investigations – Several recent epidemiological investigations of sporadic cases of human *Salmonella* infections have demonstrated that persons with antimicrobial-resistant infections are more likely to have visited or lived on a farm prior to illness onset than persons infected with antimicrobial-susceptible infections. These findings have been demonstrated in a case-control study of antimicrobial-resistant *Salmonella* Typhimurium DT104 infections (Glynn et al, 2004), and multidrug resistant *Salmonella* Newport infections (Gupta et al, 2004).

A case-control study in the United States of persons infected with fluoroquinolone-resistant *Campylobacter* also found that persons infected with fluoroquinolone-resistant *Campylobacter* were more likely to have eaten chicken or turkey than well controls. Since chicken and turkey is not imported into the United States, this finding provides evidence that poultry is an important source of domestically-acquired fluoroquinolone-resistant *Campylobacter* infections in the United States (Kassenborg et al, 2004).

(3) Field studies – Levy and colleagues conducted prospective field experiments to demonstrate how antimicrobial use in food animals selects for the emergence and disseminations of antimicrobial-resistant determinants. They found that the tetracycline resistance among *Escherichia coli* in fecal samples from chickens increased within one week of introduction of animal feed containing tetracycline. Importantly, as long as the chickens were feed animal feed containing tetracycline, the proportion of tetracycline-resistant intestinal coliforms was also increased among members of the immediate farm family, and remained higher than intestinal coliforms from neighborhood controls. (Levy et al, 1976)

Because streptothricin antimicrobial agents had not been used either in human or in veterinary medicine, the introduction of nourseothricin, a novel streptothricin antimicrobial agent, into swine production as a growth promoter in East Germany demonstrated the ability of antimicrobial growth promoters to select for the emergence antimicrobial resistance in bacteria of pigs, dissemination of resistant bacteria to humans, and horizontal transfer of the resistant determinants to other human bacteria, including pathogens. Shortly after nourseothricin use as a growth promoter in pigs, coliform organisms containing plasmids conferring nourseothricin resistance were frequently found in fecal isolates of pigs and employees of the pig farms. Within two years, similar coliform organisms with plasmids carrying nourseothricin-resistant determinants were found among family members of the employees of the pig farms, and in outpatients in adjacent communities (Hummel et al, 1986). Nourseothricin resistance was subsequently detected in human *Salmonella* and *Shigella* isolates (Witte et al, 2000). Since *Shigella* is a pathogen of primates and is not found in the intestinal tract of swine, these events provide important evidence of emergence of nourseothricin resistance in the intestinal tract in treated pigs, transfer of nourseothricin-resistant bacteria to humans, and horizontal transfer of nourseothricin-resistant determinants within the intestinal tract of humans.

(4) Case reports – There are several individual case reports of farmers, members of their families, or other persons that have become directly exposed to antimicrobial-resistant bacteria from food animals. For example, the first reported case of domestically-acquired ceftriaxone-resistant *Salmonella* in the United States involved the child of a veterinarian. Before the child's illness, the father was treating several herds for *Salmonella*. Ceftriaxone-resistant and ceftriaxone-susceptible *Salmonella* were isolated from ill cattle treated by the veterinarian. These isolates and the child's ceftriaxone-resistant isolate were indistinguishable by pulsed-field gel electrophoresis (PFGE). It appears likely that the *Salmonella* strain developed ceftriaxone resistance in the cattle and then was transmitted to the child (Fey et al, 2000).

(5) Ecological and temporal associations – In countries with surveillance data on the quantities of antimicrobial agents used in food animals, correlations have been demonstrated between the amount of antimicrobial agents used in food animals and antimicrobial resistance in selected bacteria. The lack of available data on antimicrobial use in food animals limit such comparisons.

Even in counties without surveillance on antimicrobial use in food animals, temporal associations have been demonstrated between the first approved use of an antimicrobial agent in food animals and an increase in antimicrobial resistance. In the United States, for example, there was a marked increase in the proportion of domestically-acquired *Campylobacter* infections that were fluoroquinolone-resistant following the first approved use of fluoroquinolones in food animals in 1995 (Smith et al, 1999). Similar temporal associations were observed in many European countries including the United Kingdom, and the Netherlands.

Ecological comparisons can also be made between countries that allow use of different antimicrobial agents in food animals. For example, domestically-acquired *Campylobacter* infections are commonly fluoroquinolone-resistant in European and North American countries that allow use of fluoroquinolones in food animals, but domestically-acquired *Campylobacter* infections are susceptible to fluoroquinolones in Australia which has not allowed use of fluoroquinolones in food animals (Unicomb et al, 2003).

(6) Molecular subtyping – Molecular subtyping provides important evidence of an association between use of antimicrobial agents in food animals and the antimicrobial-resistant enterococci in humans. Avoparcin, a glycopeptide antimicrobial agent, was approved for use as a growth promoter in Europe in 1974. Use of avoparcin in food animals resulted in emergence and dissemination of vancomycin-resistant enterococci (VRE) in the intestinal tract of the food animals, which was commonly transmitted to humans through the food supply, predominately via contaminated meat and poultry. Prior to the European ban on avoparcin use as a growth promoter in 1997, Europeans commonly carried VRE in their intestinal tract. Molecular subtyping of VRE isolates isolated from pigs, chickens, healthy humans from the community, and from hospitalized patients indicate genetic similarity between the isolates (Bruinsma et al, 2002).

The resistance determinants of VRE are carried on the *Tn1546* transposon. Importantly, these resistance determinants carry single nucleotide (T or G) variants. Among food animals, the G variants are found only in poultry isolates, and the T variants in swine isolates. Among VRE isolates from humans, however, the G and T variants are evenly distributed. Furthermore, human isolates from a Muslim country, where swine are not raised or consumed, carry only the G mutation. These data provide compelling evidence of an association between avoparcin use in food animals and carriage of VRE in humans (Jensen et al, 1998).

Similar molecular evidence is available to suggest an association between use of gentamicin in food animals, particularly chickens and turkeys, in the United States and high-level gentamicin-resistant enterococci in humans. When a gentamicin-resistant gene was present in resistant enterococci from animals, the gene was also present in enterococci isolates from food products of the same animal species. Furthermore, although much diversity was evident among high-level gentamicin-resistant enterococci, indistinguishable strains were found from human and pork isolates, and human and grocery store chicken isolates (Donabedian et al, 2003).

Molecular subtyping is also useful to demonstrate an association between *Salmonella* isolates from animals and humans. In an investigation of an increase of human fluoroquinolone-resistant *Salmonella* Choleraesuis infections in Taiwan, for example, molecular subtyping, including sequencing, concluded that swine were the source of the human infections; additional investigations suggest that the fluoroquinolone resistance had emerged following fluoroquinolone use in pigs (Chiu et al, 2002).

D. Human health consequences resulting from such resistance

There is accumulating evidence that antimicrobial resistance among bacteria isolated from humans resulting from with the use of antimicrobial agents in food animals results in human health consequences. These human health consequences include (1) infections that would not otherwise have occurred, and (2) increased frequency of treatment failures and increased severity of infection. Increased severity of infection includes prolonged duration of illness, increased frequency of bloodstream infections, increased hospitalization, and increased mortality.

The human health consequences the antimicrobial resistance among bacteria isolated from humans resulting from the use of antimicrobial agents in food animals have recently been reviewed (Basra, 2002). Limited evidence of these human health consequences was presented at the WHO consultations in Berlin and Geneva. Additional data has accumulated since these consultations to further illuminate these human health consequences.

(1) Infections that would not have occurred – Antimicrobial agent use in humans and food animals disturbs the microbiota of the intestinal tract, placing such individuals at increased risk of certain infections. Individuals taking an antimicrobial agent, for any reason, are therefore at increased risk of becoming infected with pathogens resistant to the antimicrobial agent. This effect has been demonstrated in case-control studies of persons infected with antimicrobial-resistant *Salmonella* in which persons exposed to antimicrobial agents for unrelated reasons, such as treatment of an upper respiratory tract infection, are at increased risk of infection with *Salmonella* that is resistant to the antimicrobial agent.

This increased risk can be expressed in the form of an “attributable fraction”; which is defined as the proportion of *Salmonella* infections that would not have occurred if the *Salmonella* were not resistant (or if the person had not been taking the antimicrobial agent for the unrelated reason). Because taking antimicrobial agents for a variety of reasons is common in the United States, antimicrobial resistance in *Salmonella* results in infections, hospitalizations, and deaths that would not have occurred in the absence of resistance. Barza and Travers (Barza et al, 2002) reviewed the literature on “attributable fraction” and concluded that antimicrobial resistance in *Salmonella* and *Campylobacter* results in 29,379 *Salmonella* infections that would not otherwise have occurred, leading to 342 hospitalization and 12 deaths, and an 17,688 *Campylobacter jejuni* infections that would not otherwise have occurred, leading to 95 hospitalizations each year in the United States.

Antimicrobial agents are commonly used in food animals, but the extent that antimicrobial resistance in *Salmonella*, *Campylobacter*, and perhaps other bacteria results in increased transmission of these bacteria between food animals that are taking antimicrobial agents has not been described. It seems likely that such use may result in increased transmission between food animals, and therefore may result in increased transmission to humans.

(2) Increased frequency of treatment failures and increased severity of infection – Increased frequency of treatment failures and increased severity of infection may be manifested by prolonged duration of illness, increased frequency of bloodstream infections, increased hospitalization, or increased mortality. Prolonged duration of illness has been demonstrated in four recent case-control studies of fluoroquinolone-resistant *Campylobacter*. In these studies, among persons treated with fluoroquinolones, the median duration of diarrhea in persons infected with fluoroquinolone-resistant *Campylobacter* was several days longer than the median duration of diarrhea in persons with susceptible infections. Two of these studies have been published (Smith et al, 1998; Neimann et al, 2003), the other two studies have been submitted to medical journals.

Prolonged duration of illness has also been demonstrated among persons infected with nalidixic acid resistant *Salmonella* Typhi treated with fluoroquinolones. Such treatment failures have been sufficiently common that several groups have suggested that the breakpoints used to define fluoroquinolone resistance in *Salmonella* and other enteric bacteria be lowered (Crump et al, 2003; Aarestrup et al, 2003).

The association between an increased frequency of antimicrobial resistance *Salmonella* and an increased frequency of hospitalization has been demonstrated in several studies. A study of 28 *Salmonella* outbreaks investigated by CDC between 1971 and 1983 found that outbreaks caused by antimicrobial-resistant *Salmonella* resulted in a greater hospitalization rate and greater case-fatality rate than outbreaks caused by susceptible infections (Holmberg et al, 1987). Recently, this analysis has been repeated on 24 *Salmonella* outbreaks investigated by CDC between 1984 and 2002. Again, outbreaks caused by antimicrobial-resistant *Salmonella* resulted in a greater hospitalization rate than outbreaks caused by susceptible infections (CDC unpublished data).

A study of 758 persons with sporadic *Salmonella* infections in 1989-1990 found that persons infected with antimicrobial-resistant isolates were more likely to be hospitalized and hospitalized longer (Lee et al, 1994).

A more comprehensive study of sporadic *Salmonella* infections has recently been completed for the Foodborne Diseases Active Surveillance Network (FoodNet) and National Antimicrobial Resistance Monitoring System (NARMS) in the United States (CDC unpublished data). Unlike the study by Lee, this analysis controlled for the serotype of *Salmonella*. Among 7,370 *Salmonella* isolates tested in NARMS from 1996-2001, *Salmonella* isolates resistant to antimicrobial agents were more frequently isolated from blood than susceptible infections. A particularly high frequency of isolation from blood was observed among isolates resistant to five or more antimicrobial agents. Among 1,415 patients interviewed, persons with *Salmonella* isolates resistant to antimicrobial agents were more frequently hospitalized with bloodstream infection than susceptible infections. Again, there was a particularly high frequency of hospitalization with bloodstream infection among persons infected with isolates resistant to five or more antimicrobial agents.

Similarly, a comprehensive study of sporadic *Salmonella* Typhimurium and *Campylobacter* infections has recently been completed in Denmark among patients with culture-confirmed infections from 1995-2000 (Staten Serum Institute, unpublished data). The Danish Civil Registry System was used to determine patient outcomes. Among 1,346 patients with *S. Typhimurium* infections, persons with nalidixic acid-resistant infections were more likely to have bloodstream infection or die in the 90 days following specimen collection than susceptible infections. Similarly, among 3,481 patients, persons with fluoroquinolone-resistant infections, or erythromycin-resistant infections, were more likely to have a bloodstream infection or die in the 90 days following specimen collection than susceptible infections.

Treatment failures resulting in death have been rare among *Salmonella* but may be expected to increase as the prevalence of resistance to clinically important antimicrobial agents increases among *Salmonella*. In the best described study of such treatment failures, an outbreak of nalidixic acid-resistant *Salmonella* Typhimurium DT104 in Denmark resulted in the hospitalization of 23 patients and two deaths. Both of the patients who died had been treated with fluoroquinolones for their *Salmonella* infections; in both instances, the coroner concluded that the fluoroquinolone resistance contributed to the deaths (Molbak et al, 1999).

A comprehensive study of mortality associated with antimicrobial resistance among *Salmonella* Typhimurium was recently conducted in Denmark among patients with culture-confirmed infections from 1995-1999 (Helms et al, 2002). Again, the Danish Civil Registry System was used to determine patient outcomes and patients were followed for two years following culture collection. To determine the increase in mortality compared to the general population, cases were matched to 10 persons from the registry by age, sex, county, and co-morbidity. Although persons with susceptible *Salmonella* infections

had a higher two year mortality than the general population, persons with resistant *Salmonella* infections had an even higher two year mortality. Furthermore, persons with nalidixic acid-resistant infections and with multidrug resistant infections had remarkably higher rate of dying in the two years following specimen collection than the general population.

E. Summary

There is clear evidence that the use of antimicrobial agents in food animals is associated with antimicrobial resistance among bacteria isolated from humans; several lines of evidence support this conclusion. Evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans is most evident among *Salmonella* and *Campylobacter*, but is also present among enterococci, *Escherichia coli* and other bacteria.

There is accumulating evidence that antimicrobial resistance among bacteria isolated from humans resulting from the use of antimicrobial agents in food animals results in human health consequences. These human health consequences include (1) infections that would not otherwise have occurred, and (2) increased frequency of treatment failures and increased severity of infection. Increased severity of infection includes prolonged duration of illness, increased frequency of bloodstream infections, increased hospitalization, and increased mortality. This accumulating evidence of human health consequences resulting from antimicrobial resistance provides documentation of the human health impact of the antimicrobial resistance among bacteria isolated from humans resulting from the use of antimicrobial agents in food animals.

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Chapter 6: Risk assessment of antimicrobial resistance from non-human use of antimicrobials

The range of adverse human health effects linked to non-human uses of antimicrobials was presented in chapter 5. This chapter reviews efforts to further characterize risk from such uses, in particular it focuses on efforts to estimate in qualitative or quantitative terms the magnitude of human health impacts attributable to non-human use.

Resistance risks to human health from non-human use of antimicrobials are inherently indirect and complex (1). Causal pathways for these risks include exposure of animals and plants to antimicrobials, selection of resistance in bacteria, movement of resistance genes among bacteria, and transfer of these bacteria through the food chain and the environment to humans where they may cause a variety of adverse health effects or outcomes (chapters 4&5). Technical and logistical constraints seriously impair direct measure of risk through epidemiological study of these complex causal pathways. Nevertheless, there is great interest in the magnitude of resistance risk as an important factor in guiding antimicrobial use policy. Therefore, a number of indirect approaches have been used to assess resistance risk. In this chapter we briefly describe publicly available human health risk assessments of non-human antimicrobial use, including their general methods and approaches, and describe their main conclusions. Some of these are summarized in Table 1. Also included are some unpublished risk assessments submitted in response to the FAO/WHO/OIE request for information. Overall limitations in existing assessments and proposed new approaches are described. No attempt, however, is made to critique the individual assessments. More extensive review of this topic, as well as critique of some assessments, is available elsewhere (1,2).

A. Review of previous international and national consultations and reports

Many expert panels have recently examined the human health risks arising from non-human antimicrobial use. These include WHO consultations (3,4) and national (5,6) or other expert committees (7,8). Reports from these panels contain information relevant to risk assessment, including descriptions of the types of resistance hazards considered important (e.g. resistance among zoonotic enteropathogens), factors contributing to the severity of the outcome (e.g. resistance to drugs important in human medicine), and the extent or prevalence of resistant bacteria in animals and food, which is relevant to exposure assessment. However, few of these reports explicitly attempt to estimate the magnitude of human health risks attributable to non-human use. The report of the 1997 WHO consultation on the medical impact if the use of antimicrobials in food animals (3) stated that “The magnitude of the medical and public health impact of antimicrobial use in food animals is not known”. The 1998 WHO consultation on the use of quinolones in food animals and potential impact on human health (4) concluded that with respect to quinolone-resistant foodborne pathogens “There has been little documented impact of this resistance on human health to date, but there is concern about the potential human health consequences if resistance were to increase and spread”. The 1999 U.S. National Academy of Sciences report (6) stated “...a significant limitation is that the real number of incidents of zoonotic antibiotic-resistant passage to humans that resolve in clinical disease might not be well documented or even tractable”. Australia’s JETACAR report (5), which used a risk assessment format, did not attempt a formal comprehensive quantitative risk assessment because of missing data, instead it proposed a format for qualitative assessment and concluded that risk assessment should be conducted on a drug-specific basis.

The 1988 Institute of Medicine report from the U.S. (7) was an early attempt to estimate quantitatively the human health impact of antimicrobial use in animals, specifically subtherapeutic penicillin or

tetracycline in animal feed. The committee determined that only for fatal salmonellosis was sufficient data available for meaningful risk assessment. The estimate of the number of people dying each year in the U.S. from resistant *Salmonella* infections of farm origin was derived as the product of five other estimates drawn from the literature and surveillance reports: (1) the annual number of cases of salmonellosis reported annually in the U.S.; (2) the fraction of human cases due to resistant *Salmonella* infections; (3) the fatality rate among cases; (4) the fraction of these deaths associated with infection of farm origin; the fraction of (4) that arose from subtherapeutic use of antimicrobials in feed. By using data from the literature and expert opinion, the authors presented in numerical terms “low”, “mid-range” and “high” values for each of the above quantities and the derived outcome to delimit the range of plausible values. They concluded that the number of people dying each year in the U.S. from resistant *Salmonella* infections of farm origin was most likely about 40, but accounting for reasonable uncertainty was somewhere between 1 and 400.

B. Other risk assessment activities conducted by individual agencies or industries

In 1999 the European Agency for the Evaluation of Medicinal Products (EMEA) published a qualitative risk assessment using *Salmonella typhimurium* and the quinolone/fluoroquinolone class of antimicrobials as a case study (8). Among the aims of the report was to use available data to answer the question: “What is the risk of adverse human health effects (in the European Union (EU)), consequent upon the development of antibiotic resistance to (fluoro)quinolones in *S. typhimurium* which is due specifically to the use of (fluoro)quinolones as veterinary medicines in farm livestock?”. The assessment was conducted by identifying a risk pathway comprising *S. typhimurium* infection in livestock, development of (fluoro)quinolones resistance in these organisms, transfer of contamination to food, infection of humans, and treatment of ill humans with (fluoro)quinolones. A series of questions pertaining to the probabilities of events at each of these stages in the pathway were developed and information available was summarized to provide qualitative answers. The principle outcome of interest was the proportion of *S. typhimurium* illnesses treated less successfully than otherwise would be the case due to resistance. The report concluded that based on available information the probability of this adverse effect was low, “... but with a high degree of uncertainty in the estimate and with much variation by country and species of livestock”.

The HAN foundation published in 2000 a report (9) whose main stated objective was “...to reassess the risk to human health caused by antimicrobial growth promoters (AGP) used as feed additives”. Steps in a proposed “risk chain” of events were described in a series of questions. Among others, these questions addressed the role of growth promoter use in resistance selection among animal bacteria, transfer of resistance to humans, and human health hazard posed by these resistant bacteria. Answers to questions posed were obtained from literature and the report stated, “Interviews with people in the field-feed producing organisations, laboratory scientists, clinical microbiologists- will be arranged.” The report did not attempt to quantify human health risk, or even to express the degree of risk in qualitative terms. Rather, it concluded “(Therefore) the proportion of antibiotic resistance within human bacteria resulting from the use of antibiotics as animal growth promoters can not be established”.

In 2000 (with revision in 2001) the U.S. Center for Veterinary Medicine, Food and Drug Administration (CVM-FDA) published a quantitative assessment of the human health impact of fluoroquinolone (FQ) resistant *Campylobacter* attributed to the consumption of chicken (10). A mathematical model was developed that multiplied various probabilities of concern. Using information from the literature (mainly epidemiological studies), foodborne disease surveillance (FOODNET) and antimicrobial resistance surveillance (NARMS), the model estimated the following quantities for a given year: (1) the

mean number of *Campylobacter* cases in the U.S. population; (2) the mean number of FQ resistant *Campylobacter* cases attributable to chicken; and, (3) the mean number of FQ -resistant *Campylobacter* cases attributable to chicken, seeking care, treated with FQ and therefore affected by the FQ resistance (the principle outcome of interest). The model further estimated the amount of FQ resistant *Campylobacter*-contaminated chicken meat consumed, which, together with (3) was used to estimate the “potential” of poultry meat contaminated with FQ -resistant *Campylobacter* to result in human illness. Human health impact was expressed in terms of the probability of being affected for various risk groups (e.g. general population, people with campylobacteriosis seeking care and prescribed antimicrobial) and the estimated number of people affected. The report estimated that in 1999 the mean number of people in the U.S. that had FQ -resistant *Campylobacter* infection and received FQ (the adverse health impact) was 9,261, with 5th and 95th percentile estimates of 5,227 and 15,326, respectively.

Angulo et al., 2000 (11) adapted the IOM risk model to estimate the human health consequences of unrestricted fluoroquinolone use in the United States. The authors estimated that antimicrobial agents, and in particular fluoroquinolones, are life-saving in approximately 2000 persons annually within invasive *Salmonella* infections. Under the assumptions that 10% of *Salmonella* isolates were fluoroquinolone-resistant and 5% of persons with invasive fluoroquinolone-resistant infections died, there would be an estimated increase of 10 deaths per year, and an increase of 100 additional deaths if 50% of strains were resistant.

In 2000 Bywater and Casewell (12) used a questionnaire to experts in the UK and elsewhere, selected on the basis of knowledge and experience in clinical microbiology, to assess the impact of antimicrobial resistance on various bacterial infections of humans, and the contribution of animal sources to the overall antimicrobial resistance problem in humans. The 20 respondents to the survey tended to perceive that the animal contribution was small (mean 3.88%).

Anderson et al published in 2001 an assessment of the impact on human health of resistant *Campylobacter jejuni* from fluoroquinolone (FQ) use in beef cattle (13). A mathematical model beginning at the retail level was developed to estimate the probability of contamination of cooked ground beef or fresh beef. The model also estimated the concentration of *C. jejuni* in contaminated product, the effects of cooking on contamination, and the probability of infection or illness given consumption of contaminated product. A second model incorporated the prevalence of FQ resistance in human strains of *C. jejuni*. The potential clinical impact of FQ resistance in *C. jejuni* was assessed by estimating the number of human cases of FQ -resistant *C. jejuni* infection from beef for which treatment with FQ was undertaken. Inputs for the model were obtained from a variety of sources, including published literature, surveillance reports and expert opinion. The model estimated that in the first year of FQ use in beef cattle in the U.S., at least 10 and as many as 80 people would have FQ-resistant infection for which another drug or treatment strategy would be required, and 1-12 of the estimated 150 hospitalized cases may not respond to FQ therapy. The model predicted that risk is greater when there are failures in food preparation, including proper cooking.

Travers and Barza, 2002 (14) used data from published epidemiological studies and national surveillance data to estimate the annual excess days of illness due to resistant *Campylobacter* infections in the U.S. Assuming an average of two additional days of illness in patients with fluoroquinolone-resistant *Campylobacter* infection and treated with a fluoroquinolone, they estimated an excess of 410,926 days of illness annually in the U.S. population attributable to antimicrobial use in animals. They also constructed a model of the number of hospitalizations and excess days of hospitalization due to foodborne *Salmonella* infection, which is similar conceptually to the IOM model. Using data from

published studies, they estimated 8677 extra days of hospitalization due to antimicrobial resistance; 90% attributable to antimicrobial use in food animals. In a companion study (15), these authors estimated the excess number of infections due to antimicrobial infections (the “etiologic fraction”). The authors conducted a random effects meta-analysis of epidemiological studies investigating increased vulnerability of infection resulting from antimicrobial treatment. They estimated that annually in the U.S., antimicrobial-resistant infections from animals leads to an additional 29,379 nontyphoidal *Salmonella* infections and an additional 17,668 *Campylobacter* infections.

In 2002, Cox and Popken reported an assessment of human health risks from virginiamycin (VM) use in chickens (16). The human health impact of interest was quinupristin-dalfopristin (QD) treatment failure among vancomycin-resistant *Enterococcus faecium* (VRE) that were also resistant to QD. The basic analytical strategy was to estimate the proportion of QD-resistant VRE-infected patients whose infection is attributable to VM use in chickens. A mathematical model was developed that estimated the frequency of the following: (1) VRE infection; (2) proportion of VRE resistant to QD; (3) proportion of QD-VRE from chicken; (4) prescription of QD to patient; and (5) QD therapy fails due to QD resistance. The model inputs were based on surveillance data from Australia and the U.S. and published literature. The model estimated that a VM ban in Australia would reduce treatment failures by an average of 0.35×10^{-3} cases, and mortalities by an average of 5.8×10^{-5} for the population over a 5-year period. In the U.S., a VM ban was predicted to decrease VM treatment failures by an average of 1.8 cases, and mortalities by an average of 0.28 cases in the population over 5 years. In 2003 these authors prepared two additional reports for the U.S. Animal Health Institute (AHI) that were related to human health risk assessment of non-human antimicrobial use. One report (17) is an overview of risk analysis practices and procedures related to antimicrobial feed additives, and the other is a description of a proposed “risk rating technique” (18).

The Australian Pesticides & Veterinary Medicines Authority published in 2003 the results of a qualitative human health risk assessment of virginiamycin use in livestock (19). Consideration was given to the probability of disease in humans due to infection with resistant pathogens arising from use of virginiamycin in animals, as well as the consequences of disease. Within the Australian context, the following general matters that bear on probability of infection were addressed in the assessment: factors related to streptogramins (e.g. mode of action, mechanisms of resistance), patterns of streptogramin use in humans and animals, bacterial species likely to be affected, national and international resistance and cross-resistance data from human and animal isolates, data on co-selection, volume of streptogramins used, and likely routes of transmission. The assessment of impact on human health considered the importance of streptogramins in human medicine, the importance of enterococcal infections in humans, and the impacts of treatment failures. An assessment of uncertainty was also made. The report concluded that the probability of human disease due to exposure of streptogramin resistant *E. faecium* of animal origin is low and the severity of impact in susceptible humans is high, but the severity of impact in the general population is low.

Smith et al., 2003 (20) used a compartmental mathematical model to qualitatively assess risks of emergence of streptogramin resistance in *Enterococcus faecium* associated with virginiamycin use as a growth promoter. Specifically, they used the model to explore the conditions under which virginiamycin use in animals increases the fraction of patients colonized by streptogramin-resistant *E. faecium* (SREF). The general structure of the model included exposure of people to SREF, a fraction of which is the result of virginiamycin use (this fraction is an important and uncertain model parameter), colonization or transient carriage, spread of infection person to person and through hospitals, disturbance of enteric microflora by antimicrobial treatment, streptogramin treatment, and other steps. The model

was used to investigate a number of plausible scenarios resulting in epidemics (involving continued person to person spread), quasi-epidemics, and non-epidemics. The authors conclude that emergence of SREF is most likely to result from an interaction between streptogramin use in humans and virginiamycin use in animals.

In 2003, the E.C. published an opinion on human health risk caused by use of fluoroquinolones (FQ) in animals (21). While information relevant to a risk assessment was reviewed, no attempt was made to quantify or otherwise estimate the magnitude of the human health impacts attributable to resistance.

In 2003, risk assessments of macrolide (tylosin or tilmicosin) use in fed cattle, poultry and swine, on the treatment of human foodborne disease were reported (22,23). In these assessments, the adverse effects of interest were human illnesses caused by macrolide-resistant *Campylobacter* or macrolide-resistant *Enterococcus faecium* (hazardous agents) and treated with a human antimicrobial from the macrolide class. Risk was modelled as the yearly probability that an average person in the U.S. population would experience an adverse therapeutic event (e.g. longer duration of diarrhea, progression to more severe disease, or mortality) from a meal that originated from a contaminated carcass. The models used estimated the following probabilities or frequencies: (1) macrolide administration to animals (this assessment used survey-based estimates of the frequency of food animal treatment with macrolides); (2) hazardous agent selected above background level; (3) hazardous agent escapes from the farm; (4) hazardous agent remains on carcass after harvest; (5) hazardous agent survives to retail meat; (6) contaminated product is mishandled and presented to consumer; (7) consumer becomes ill; (8) patient treated with macrolide; and (9) macrolide treatment failure. Input data for the model were obtained from approved antimicrobial label claims, published literature and U.S. government surveys. The beef cattle model estimated that the annual probability of an adverse health event in the U.S. due to treatment of a human foodborne infection caused by a hazardous agent of interest was <1 in 236 million for *Campylobacter* and <1 in 29 billion for *E. faecium*. The equivalent estimates for poultry were <1 in 14 million for *Campylobacter* and <1 in 3 billion for *E. faecium*, and for swine were <1 in 53 million for *Campylobacter* and <1 in 21 billion for *E. faecium*.

C. Analysis: trends in risk assessment of non-human antimicrobial use and proposed new strategies

Relatively few studies have attempted to estimate, in either qualitative or quantitative terms, the magnitude of antimicrobial resistance public health impacts due to non-human antimicrobial use. Given the importance of this subject to risk management and other policy decisions, the paucity of such studies reflects the difficulties encountered in trying to characterize these risks.

It is beyond the scope of this document to undertake a thorough analysis of the quality, accuracy, significance and credibility of the available risk assessments. In general, however, the reports vary widely in the thoroughness and clarity with which they describe their methods, identify and deal with uncertainties, qualify their findings and declare their assumptions. Collectively, these assessments do not produce “definitive” findings, and will not end debate over the magnitude of public health impacts from non-human uses of antimicrobials. Nevertheless, the actual estimates of human health impact that were generated may be useful in some cases. Furthermore, there are other positive contributions that have been made: they provide experience with risk assessment that may help to improve future analyses, they identify some additional knowledge gaps, and they may help some stakeholders better understand the complexity of the resistance problem.

A few assessments were limited to qualitative or semi-quantitative estimates of risk (8,9,12,21). In some cases this appears to reflect the lack of quantitative data needed to address the questions posed, while in others it reflects the nature of the questions asked and the techniques used. Several quantitative assessments (10,11,14) adopted the general approach first used by the Institute of Medicine (IOM) in 1988 (7); to use national surveillance data on foodborne disease and antimicrobial resistance, supplemented with more focused survey or research data, to estimate the total number of cases of human illness in a population, and then estimate the proportion resistant to antimicrobials, and then the proportion of these due to use of antimicrobials in animals. This could be called a “top-down” approach because it starts with measurement of human disease incidence. Its main advantages are comparative analytic simplicity, need for fewer assumptions and fewer data than more complex models. On the other hand, problems are likely to be encountered when using this approach to estimate the impact of potential risk reduction strategies at various levels of the food-chain, and to assess potential hazards, such as resistance effects of drugs not yet on the market. Furthermore, most countries lack sufficient surveillance data for this approach. The main alternative, the “bottom-up” or “farm-to-fork” approach used in some assessments (13,22,23), attempts to model microbial infection, spread, and acquisition of resistance through animal production, and then harvesting, processing and preparation of various animal-derived food products, eventually leading to consumption by humans. This approach compensates for some of the disadvantages of the “top-down” approach, but is usually more complex and vulnerable to data gaps (given the complexity of the food production and processing chain), may require very complex mathematical procedures and expertise, may require antimicrobial use information, and often requires more assumptions.

The quantitative risk assessments available do not fully address the broad range of potential human health impacts, or the spectrum of antimicrobials and organisms relevant to a comprehensive assessment of risk. Instead, they focus on specific antimicrobial/bacteria combinations that have been or continue to be of special interest to industry or government. Bailar and Travers (1) made some general observations of antimicrobial resistance risk assessments that are also applicable here: existing assessments focus on few specific clinical outcomes, few species of bacteria and few animal species; they do not consider a more general shift toward more resistant bacterial populations (e.g. resistance transfer across bacterial species, issues of co-selection or cumulative effects in bacterial populations); and they focus on what has already happened, which may not predict future risk.

Many of the individuals or groups that have undertaken or commissioned risk assessments later proposed alternative strategies or frameworks for future assessments. This is an indication that those actually conducting the assessments were not particularly satisfied with the methods available.

In 2001, an OIE ad hoc group of experts proposed an approach to qualitative / quantitative risk assessment for antimicrobial resistance (24). The proposed process is divided into release assessment, exposure assessment, consequence assessment and risk estimation. In the OIE code, release assessment was defined as “Description of the biological pathways necessary for the use of an antimicrobial in animals to release resistant bacteria or resistance determinants into a particular environment, and estimating the probability of that complete process occurring either qualitatively or quantitatively”; exposure assessment was defined as “Describing the biological pathways necessary for exposure of animals and humans to the hazards released from a given source, and estimating the probability of the exposure occurring, either qualitatively or quantitatively”; consequence assessment was defined as “Description of the relationship between specified exposures to a biological agent and the consequences of those exposures.”; and risk estimation was defined as “Integration of the results from the release assessment, exposure assessment, and consequence assessment to produce overall measures of risks

associated with the hazards identified at the outset”. The report further explains the rationale and approach to qualitative and quantitative assessment and the integration of risk assessment, risk management and risk communication, into the larger domain of risk analysis.

Salisbury et al (25) proposed an alternative approach to assessment of resistance risks that appears to have arisen, at least in part, from Australia’s JETACAR report (5). In this approach, three separate assessments are conducted, one for each of three hazards: the antimicrobial, the resistant bacteria, and the genetic determinants for resistance. Three adverse outcomes were identified as: emergence of resistant bacteria, spread of resistant bacteria, resulting in human exposure/ infection, and transfer of resistance genes to other bacteria. It was envisaged that the data requirements for risk assessment of the three types of hazards would differ: data for risk assessment of antimicrobial use is equivalent to that for other chemical use; data for risk assessment of spread of resistant bacteria is equivalent to microbial risk assessment; and the data for risk assessment of genetic determinants of resistance is equivalent to a genetic risk assessment. The results of these three assessments are combined in an overall risk characterization.

After reviewing a number of antimicrobial resistance risk assessments, Bailar and Travers (1) identified a number of desirable characteristics of a new approach: reduced demand on resources; common format; reduced demands for data; easy comprehension by non-experts; and ready adaptation. They concluded that a multiplicative model (similar to the “top-down” approach described above) could meet these criteria, and that such a model should contain the following four estimates: (1) annual number of symptomatic infections by the organism of interest in a specific risk assessment; (2) fraction of those occurrences in which the bacterial strain was clinically resistant to the antimicrobial or class of antimicrobials under study; (3) annual number of occurrences in which infection by a resistant strain led to the specific outcome under study; and (4) fraction of the above outcomes in which the antimicrobial resistance was a result of the farm use or category of uses under study.

In 2002 (and modified in 2003), CVM-FDA published a recommended approach for assessing resistance risks in new animal drug applications (26). A qualitative approach is recommended, although the possibility of quantitative assessments is not excluded. As a first and distinct step, FDA recommends the drug sponsors conduct a “hazard characterization” in order to determine the type of information needed for risk assessment, or whether a risk assessment is actually needed. This step includes preliminary consideration of a variety of drug and resistance information. If it is determined that a risk assessment is needed, the recommended steps conform to the OIE format described above (21). The main outcome of the risk assessment is the risk estimation, which provides “...a qualitative indication of the potential risk to human health of the proposed use of the antimicrobial new animal drug”. Applicants are required to submit information related to the main categories of the assessment and provide information relevant to ranking the drug as high, medium or low in most of the categories. For the release assessment, this includes among other factors, the drug’s spectrum of activity, resistance mechanisms, and resistance transfer. In the face of uncertainty, conservative rankings are used. For exposure assessment, ranking considers frequency of bacterial contamination (e.g. *Salmonella*) of food products, and *per capita* consumption of animal-derived food categories from treated animals. Ranking is achieved based on an integration of the ranking of probability of human exposure through food with the ranking of consumption of the animal-derived food. The consequence assessment involves ranking the drug into “critically important”, “highly important” and “important” based on the usefulness of the drug in foodborne infections, availability of alternative therapies, the ease with which resistance develops and other factors. The risk estimation is the integration of the previous steps, and results in ranking of drugs as high, medium or low risk to human health. FDA proposes to use the information provided by

the sponsor and the results of the risk assessment to determine the applicable antimicrobial risk management steps.

Australia and the E.U. have also published proposed or draft guidelines for assessing resistance risks that appear to be based on a qualitative approach (27,28). In future, it is not clear how risk assessment approaches for new antimicrobial drug submissions will compare with those for existing drugs. In many countries the drug submission process, including data submitted in risk assessment, is considered confidential. This may act as a barrier to sharing of relevant information and to continued evolution of risk assessment methods. Risk assessments are likely to be internationally relevant only to the extent that the information used is broadly applicable. While this may hold for most drug-related factors, for example, pharmacology, pharmacokinetics, importance of drugs to humans, etc., many other factors are likely to vary among countries, including prevailing farming conditions, pathogen load in foods, food consumption patterns, etc. Ideally, risk assessment methods should be flexible enough to accommodate such differences.

D. Summary/conclusions/recommendations

A modest number of studies and reports attempted to estimate the magnitude of public health impact from non-human uses of antimicrobials. Most of these have been conducted in the last six years, since the WHO meeting in Berlin (3). Some of these estimates were qualitative (e.g. high, medium, low) and others were quantitative. A variety of risk assessment outcomes were used, for example, the annual number of mortalities, and annual number of cases of illness due to resistant infections treated with antimicrobials. Estimates of impact and attendant uncertainty ranged widely, depending on the microbial agent, antimicrobial drug and animal species included in the risk assessment models.

Several different methods for risk assessment were used, including “top-down” approaches, starting with population estimates of illness, and “bottom-up” or “farm-to-fork” approaches. In addition, new or modified approaches have been proposed. Although existing risk assessments were not critically reviewed for this report, they appear to be highly variable in terms of rigour, clarity of methodological description, assumptions made, and handling of uncertainty. Some of the assessments may have been valuable in supporting risk management policy, and others have been useful in providing better understanding of resistance issues and showing data gaps and research needs. Before any are used for policy decision, however, they need to be carefully reviewed. Improved methods for critical appraisal of resistance risk assessments are needed to help guide their use.

Existing risk assessments do not adequately address the broad range of potential human health impacts, or the spectrum of antimicrobials and organisms relevant to a comprehensive assessment of risk. They do not explicitly consider global spread of resistant bacteria, spread of resistance genes between bacteria or the cumulative effects of resistance in populations. However, formal risk assessment of antimicrobial resistance is a fairly new activity. It should be expected that the process would improve as experience is gained, methods evolve, and the biology of resistance is better understood. It is likely that a variety of methods for assessing risk will be needed, including methods applicable to new drug submissions, and methods for review of existing drugs.

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Table 1. Some risk assessments related to the use of antimicrobials in food animals and its effects on human health

Reference	Model	Animal Species	Antimicrobial	Microorganism	Adverse Effect	Risk
IOM 1989 (7)	Quantitative Estimate of annual number of salmonellosis deaths from subtherapeutic use of antimicrobial	Food animals	Penicillin and tetracycline	<i>Salmonella</i>	Mortality	Most likely 40 fatalities per annum
Wooldridge EMEA-CVMP 1999 (8) Europe	Qualitative “Farm-to-fork” plus incidence of adverse effect	All farm livestock	Fluoroquinolones (FQ)	<i>Salmonella Typhimurium FQ resistant</i>	Adverse human health effects due to development of resistance due to use of quinolones in animals	Low risk
FDA 2000 (10) USA	Quantitative Links human cases with contaminated chicken	Chicken	Fluoroquinolones (FQ)	Campylobacter	Failure of treatment of human campylobacter infections with FQ	As a result of FQ use in broilers, mean estimate 9,261 cases in 1999. Alternatively expressed risk as probability of 1 in 19 among people with campylobacteriosis seeking care and prescribed antimicrobial in 1999.

Reference	Model	Animal Species	Antimicrobial	Microorganism	Adverse Effect	Risk
Anderson et al 2001 (12) USA	Quantitative “farm to fork” plus response to treatment of humans	Beef cattle Fresh beef Ground beef	Fluoroquinolones (FQ)	<i>Campylobacter jejuni</i>	Failure of treatment of human campylobacter infections with FQ Death	After 1 year of FQ use in beef cattle, 12 cases of failure of FQ treatment in humans from FQ-resistant campylobacter in ground beef, 44 cases after 10 years 1 FQ-resistance-associated death in 10 years
Cox & Popken, 2003 (13) Also Cox & Bafundo 2002 Australia & USA	Quantitative Response to treatment in humans plus molecular genetics of chicken strains of <i>E. faecium</i>	Chicken	Virginiamycin (Streptogramin SG resistance)	<i>Enterococcus faecium</i> SG resistant	Effect of virginiamycin ban on human treatment failure and mortality from use of quinupristin/dalfopristin	Australia: 0.35×10^{-3} failures, 5.8×10^{-5} deaths avoided in 5 years USA: 1.8 failures, 0.28 deaths avoided in 5 years In Australia plus USA, “0-<1 life saved in 5 years as a result of a ban”
AVPMA 2003 (16) Australia	Qualitative	Cattle, sheep, pigs, poultry	Virginiamycin (Streptogramin SG resistance)	<i>Enterococcus faecium</i> SG resistant	Failure of treatment of <i>E. faecium</i> infections in humans	Low risk But high impact in susceptible humans

Reference	Model	Animal Species	Antimicrobial	Microorganism	Adverse Effect	Risk
Smith et al 2003 (17)	Semiquantitative	Food animals	Virginiamycin (Streptogramin SG resistance)	<i>Enterococcus faecium</i> SG resistant	Epidemics	Emergence of streptogramin – resistant <i>E. faecium</i> most likely from interaction between streptogramin use in humans and virginiamycin use in animals
Hurd et al 2003 (19) & Doores et al 2003 (20) USA	Semiquantitative Binomial event-fault tree	Cattle, Poultry Swine	Tylosin Tilmicosin (Macrolide)	<i>Campylobacter</i> <i>Enterococcus faecium</i>	Failure of treatment with erythromycin in humans	Range from <1 in 14×10^6 failures yearly to <1 in 29×10^9 failures yearly from a contaminated meal

Chapter 7: Assessment procedures used by the joint expert committee on food additives and contaminants (JECFA) in the safety evaluations of veterinary antimicrobial residues in food to determine the effects on the human intestinal microflora

A. INTRODUCTION

Antimicrobials are used in animal husbandry for therapy to treat disease, control and prevent infection, and growth promotion. In most developed countries, national regulatory agencies and international committees evaluate the safety of drugs used in food animals for potential human health risks based on all available data on chemical, pharmacological, and toxicological properties of veterinary drugs derived from studies of experimental animals and observations in humans. There are two safety evaluations unique to antimicrobials: 1) safety of antimicrobial agent use in animals leading to development of antimicrobial resistant bacteria and resistance determinants which could spread in bacteria to humans via the food chain or zoonotic spread to humans and 2) safety of ingestion of drug residue in foodstuffs (meat, milk, eggs and edible tissue) in terms of effects on human intestinal flora. A third microbiological impact evaluated only in Europe concerns the potential effect of drug residue in milk, on milk fermentations for yoghurt, cheeses, and other products used in the dairy industry. While development of guidelines to address the spread of resistance has only recently been put in place (USFDA Guidance 152, EU Guideline EMEA/ CVMP/244/01, JETACAR Guidance part 10 in Australia, and VICH Draft Guidance 27), guidance for addressing microbiological safety of ingested residue has been in place for a number of years. Antimicrobial residue is a compound present in edible or target tissue of a food-producing animal, either from intentional or inadvertent introduction of the compound itself, its metabolite, and other substances that form in or on the food after introduction of the compound. Historically, agencies have used different regulatory approaches to examine the safety of residue ingestion. The vast differences in approaches taken by the various agencies have resulted in formation of an international committee (VICH) to review methodologies currently used for these evaluations, and make recommendations regarding their applicability in attempts to support harmonization of regulatory approaches worldwide.

B. Overview of safety evaluation of drugs administered to food animals

Various organizations and regulatory authorities have developed methods and adopted regulatory approaches to evaluate the safety of edible foodstuffs (milk, meat, eggs, and edible tissue such as fat, kidney, and liver) derived from animals treated with a specific drug. While regulatory approaches vary among international authorities and national agencies, objectives encompass three basic evaluations and decisions: 1) safe ingestion level quantified in terms of an acceptable daily intake (ADI) for consumption of residues for the lifetime of an individual; 2) maximum residue levels (MRL) allowable in all edible foodstuffs derived from treated animals to be consumed by humans and 3) withdrawal time needed after the drug is administered for residues to fall below the MRL so animals may be slaughtered for subsequent processing and consumption.

The ADI is based on an array of toxicological safety evaluations taking into account acute and long term exposure to the drug and its potential impact, such as carcinogenicity, genotoxicity, reproductive toxicity, teratology, neurotoxicity, immunotoxicity, allergenicity, ocular toxicity, cardiac toxicity and, in the case of antimicrobial agents, the safety for gastrointestinal microflora. The ADI is determined as a

conservative estimate of the safety ingestion levels by the human population, based on the lowest "no effect level" (NOEL) among a battery of toxicological safety studies.

The ADI provides the basis for determining the MRL of the drug in treated animals. As is the case for ADI determination, the regulatory approach used to assign MRL's to various edible tissues is dependent on the regulatory agency and is beyond the scope of this report. Basically, approaches take into consideration how much of a foodstuff derived from an animal may be consumed on a daily basis. Typically the daily intake estimate is high. For example, in the case of the Joint Expert Committee on Food Additives (JECFA) and European Agency for the Evaluation of Medicinal Products Committee for Veterinary Medicinal Products (EMEA CVMP), they assume that every day an average person consumes 300 g of muscle, 100 g liver, 50 g kidney, 50 g fat, and 1.5 liters of milk, from treated animals. Using this assumption, the MRL for each foodstuff is set so that, if a person were to consume this entire "food basket" of foodstuffs (each foodstuff having the respective MRL) from the animal, total consumption of residue would be below the ADI. The MRL for all foodstuffs is set based on the assumption that an individual consumes foodstuffs from an animal containing the MRL, and that total residue consumption is still at or below the ADI. Agencies also establish a legal drug withdrawal time to assure that animals are slaughtered at or after drug residues in the tissues are below the MRL for each.

C. Importance of human intestinal microflora

Human intestinal flora is a balanced ecosystem that is very important in maintaining an individual's health. It is well established that microflora in the human gastrointestinal tract forms an extremely complex, yet relatively stable, ecological community populated with over 10^{11} bacterial cells per gram of content and contains more than 400 bacterial species (Carman et al., 1993, Drasar & Duerden, 1991, Moore & Holdeman, 1974). This high bacterial concentration accounts for about 30% of the fecal mass. Approximately 90% of these species are obligate anaerobes consisting of 30 different species. The predominant genera are *Bacteroides spp.*, *Eubacterium spp.*, *Bifidobacterium spp.*, *Clostridium spp.*, *Fusobacterium spp.*, *Ruminococcus spp.*, *Enterococcus spp.*, *Peptococcus spp.*, and *Peptostreptococcus spp.* The predominant bacteria are obligate anaerobes since the lower regions of the gastrointestinal tract form a highly reducing environment with a redox potential of -200 to -350 mv. Among the facultative anaerobic bacteria, the most commonly isolated species in feces is *Escherichia coli* which belongs to the family Enterobacteriaceae and accounts for approximately 1% of the fecal flora. Although there may be large individual variations in the proportions of major species from person to person depending on the diet, population sizes of different species from the same individual are stable (Moore & Holdeman, 1974, Moore & Moore, 1995). The microflora interacts with its host both locally, due to its intimate contact with the intestinal mucosa, and systemically influencing diverse responses and immunological, physiological, anatomical, metabolic, nutritional and toxicological functions. Intestinal microflora are an essential component of human physiology because they act as a barrier against colonization of the gastrointestinal tract by pathogenic bacteria (Vollaard & Clasener, 1994). They also play important roles in digestion of dietary components, metabolism of drugs, xenobiotics and nutrients, and providing compounds such as short chain fatty acids and other essential nutrients that are later absorbed into the system (Chadwick et al., 1992).

Although the microbial population in the gastrointestinal tract is generally stable, clinical studies have shown that therapeutic doses of antimicrobials may change the balance. Oral exposure to antimicrobials that are poorly or incompletely absorbed, excreted in the bile, or reach the intestine through circulation

and excretions from intestinal mucosa can potentially alter the ecology of the intestinal microflora (Carman et al., 1993, Edlund & Nord, 1999, Finegold et al., 1983). The type or extent of change in the system will depend on the spectrum of action of the antimicrobial drug, its dose, the length of an individual's exposure to the drug, as well as the bioavailability, metabolism, distribution in the body and route of excretion. The lowest concentration of any antimicrobial drug that does not affect the intestinal flora has not been examined to any great extent in published literature, thus making work by the agencies less than straightforward. However, studies using *in vitro* (continuous or semi-continuous flow culture systems) and *in vivo* human flora-associated rodent test systems and human volunteers have shown that therapeutic levels of antimicrobial drugs are capable of altering different parameters of intestinal flora depending on the spectrum of action and concentration of a drug (Edlund & Nord, 1999, Finegold et al., 1983, Gorbach 1993, Heimdahl et al., 1985).

The main concerns of adverse effects of antimicrobial drug residues on human intestinal flora are selection of resistant bacteria and disruption of the colonization barrier (or barrier effect) of resident intestinal flora. Colonization barrier or barrier effect is the "limiting action" of normal flora on colonization of the bowel by exogenous or indigenous potentially pathogenic microorganisms (Vollaard et al., 1994). Other effects, such as alteration of metabolic activity of the flora may also be important. However, there is no documented evidence that antimicrobials cause human health effects (e.g., prolonged antimicrobial therapy, prolonged hospital stay, predisposition to infection, treatment failure) when present as residue concentrations approved as safe by regulatory agencies. The failure to find recorded adverse health effects does not negate the human health concern and regulatory agencies, accordingly, have put into place requirements for a safety assessment of veterinary antimicrobial residues in food to mitigate the risk.

D. Methods for measuring the effects of antimicrobial compounds on human intestinal microflora

Cerniglia and Kotarski (1999) reviewed the variety of *in vitro* and *in vivo* experimental test systems to study the effects of antimicrobial residues on the human gastrointestinal tract (Table 1). The point that should be made is that although a variety of *in vitro* and *in vivo* experimental test systems and approaches have been and can be used to assess the safety of veterinary drug residues in foods for human consumption, none has been validated in accordance with the procedures proposed by the Interagency Coordinating Committee on the Validation of Alternative Methods, wherein the observed endpoints are validated to predict the biological impact they intend to measure, and that the test methods should provide repeatable results under standardized experimental procedures as confirmed by different laboratories. As noted above and in the review by Cerniglia and Kotarski (1999), *in vivo* and *in vitro* test methodologies for characterizing the nature of antimicrobial effects on intestinal flora have been developed, but few studies are available as a basis for determining a NOEL of antimicrobials. More studies are needed to address the variability of protocols to test the effect of low levels of antimicrobials on intestinal microflora and their relevance to human exposure before an appropriate design can be used in test validation. Further, none of these methods have been evaluated for their prediction of human health impact.

E. International and regulatory approaches in assessment of the effects of antimicrobial drug residues from food of animal origin on human intestinal flora

The requirement for drug sponsors to account for the potential impact of antimicrobial drug residue on ingestion on human intestinal flora first began in 1986 as a requirement of the drug registration or re-registration process. The U.S. Food and Drug Administration Center for Veterinary Medicine (USFDA CVM, 1996, 2001, 2003), FAO/WHO (1995, 1998, 2000), EMEA/CVMP(1995, 2001, 2002, 2003) have since issued and updated guidance's reflecting the changing status, testing experience and increasing emphasis these agencies have placed on this evaluation over the years.

Although the effect of antimicrobial residues in food on human intestinal microflora has been a concern for many years, a harmonized approach to determine the threshold dose that might adversely disturb the flora has not been established for the different classes of antimicrobial drugs. This is due in large part to the lack of validated test systems available to measure a NOEL for drug concentrations against the colonization barrier and resistance emergence. Currently the Veterinary International Cooperation and Harmonization (VICH) Safety Working Group is developing a unified approach in evaluating data to determine the impact of veterinary antimicrobial drug residues in food on the human intestinal microflora (VICH, 2001, 2002).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is responsible for the safety assessment of veterinary drugs in foods and has been charged with advising and providing guidance to FAO and WHO member states and to the Codex Alimentarius Commission (CAC) on four broad tasks: a) establish and further elaborate principles for evaluating the safety of residues of veterinary drugs in foods and determining acceptable and safe levels of residues when drugs are administered to food producing animals in accordance with good practice in the use of veterinary drugs; b) determine criteria for appropriate methods of analysis for detecting or quantitating residues of veterinary drugs in foods; c) evaluate or re-evaluate the safety of residues of certain veterinary drugs; d) discuss and provide advice on matters of interest arising from reports of sessions of the Codex Committee on residues of veterinary foods.

Each national regulatory agency and JECFA has scientific experts who provide advice on the safety of veterinary drug residues and appropriate studies to determine their safety. The scientific advisory groups make recommendations that will later become standards when approved by the organizations. CAC sets standards for veterinary drug residues based on recommendations made by JECFA through the Codex Committee on Residues of Veterinary Drugs in Food. The EMEA sets standards based on recommendations from the Committee for Veterinary Medicinal Products (CVMP). The VICH recommends requirements and protocols for determining human food safety of veterinary drugs based on recommendations from the Safety Working Group (SWG).

JECFA initially addressed the microbiological safety of veterinary drug residues in foods in June of 1987. The Committee concluded that the antimicrobial properties of veterinary drug residues would become the determining factor in safety evaluation when the toxicity of the substance is so low that their residues could be tolerated without any withdrawal period. In such a case, the safety of the residues would be based on their danger to human health due to their selective pressure on intestinal microflora favoring growth of microorganisms with natural or acquired resistance (FAO/WHO, 1988).

In 1990, the Committee concluded that the most important characteristics of intestinal microflora to be considered when assessing the microbiological risk of antibiotic residues in food are the proportion of anaerobic bacteria, stability of the flora, and the barrier effect. Thus, the Committee determined that the safety evaluation of antimicrobial residues should be based on data related to bacteria that constitutes the flora, taking into account the barrier effect. If human data are not available, animal studies might be considered. The Committee encouraged the validation of animal models such as germ-free rodents implanted with human intestinal microflora and also concluded that in the absence of *in vivo* data, *in vitro* data such as minimum inhibitory concentrations (MIC) could be used on a temporary basis for safety evaluations (FAO/WHO, 1990).

In 1991 for the first time, JECFA calculated the ADI for an antimicrobial drug (spiramycin) using MIC data from four species of the dominant anaerobic flora. A formula was developed using the modal MIC of the bacteria tested, safety factors to cover different variables, the daily fecal bolus, the fraction of oral dose available, and the weight of humans (FAO/WHO, 1991).

In 1994, JECFA concluded that the evidence of risk due to low levels of antimicrobial residues is minimal and other methods for studying microbiological endpoints may be useful for assessing this risk. MIC data would continue to be accepted for determining ADIs until other methods could be developed and accepted for this purpose (FAO/WHO, 1995).

In 1995, JECFA discussed a new 'decision tree' approach (Table 2) to the safety evaluation of antimicrobial residues (FAO/WHO, 1996), and in 1996, the Committee concluded that more research was needed concerning the public health risk of antimicrobial residues and their effects on human intestinal microflora. They recommended that MIC data should not be the only method used to calculate an ADI and that data from *in vitro* or *in vivo* model systems or any other relevant data should be used for setting ADIs. In the absence of human data, data from *in vivo* model systems (e.g., human flora-associated rodents) or *in vitro* models (e.g., continuous flow cultures) could be used for determining ADIs for antimicrobial drugs. They recognized the limitations of the formula method, and the formula using MIC data was again modified.

JECFA formula to derive an ADI =
$$\frac{\text{MIC}_{50} \text{ (mg/g)} \times \text{Mass of colonic contents (220g)}}{\text{Fraction of oral} \times \text{Safety factor} \times \text{Weight of human (kg) dose bioavailable}}$$

Safety factor: takes into account the uncertainty about the amount and relevance of data available for review. It may range from 1 to 10. A value of 1 is used when extensive relevant microbiological data are provided.

The Committee recommended development and validation of *in vitro* and *in vivo* model systems that would be more appropriate for determining NOELs and setting ADIs for antimicrobial residues.

In 1998, the Committee determined the ADI of several antimicrobial agents (gentamicin, sarafloxacin, tetracyclines) based on effects on *in vitro* studies and using the formula approved in their 47th meeting (FAO/WHO, 1998). Tetracycline's ADI was determined based on development of resistant *E. coli* seen in a human study and also confirmed in a continuous flow thermostat study (FAO/WHO, 1999). In February 2000, the Committee determined the ADI for lincomycin using the 'decision tree' approach (reproduced in Table 2) discussed in 1995. Since then, several other antimicrobials were reviewed using the 'decision tree' approach, namely cefuroxime and neomycin. Since toxicological evaluations

historically result in trace residues, the first three questions of the decision tree are intended to determine whether microbiologically active drug residue will even enter the colon of an individual if the person ingested the ADI limit based on other toxicological testing. To address these first three questions, the JECFA Expert Group uses data provided by the sponsor and the literature to determine whether the drug is microbiologically active, whether the drug's absorption and metabolism characteristics, and at ingestion rates based on the acceptable daily limit (as determined from all other toxicological studies) will lead to any microbiologically active drug residue entering the gut tract. If the answer is no and the Committee can use the data to show that microbiologically active residue does not enter the colon, then the ADI is not based on microbiological endpoints and the ADI derived from other toxicological studies is assumed to address the concern of impact on microbiological residue.

However if the answer is yes to any of the first three questions, all published literature and data provided by the sponsor regarding characteristics of the drug and related classes are used to address question four to determine whether the ADI derived from toxicological data is sufficiently low to protect the intestinal microflora. If the ADI is not sufficient, then available information about the drug and drug class are used to identify effects which could occur in gastrointestinal microflora (question five), and if no information is available, then specific studies using an *in vitro* or an *in vivo* test system are conducted to determine the most sensitive adverse effect(s) of the antimicrobial on human intestinal microflora. Adverse effects of human health concerns to be considered are disruption of the colonization barrier (barrier effect, the selection of resistant bacteria in the colon, and change in metabolic activity of intestinal microflora).

The barrier effects (or colonization resistance) are the property of the flora that prevents overgrowth of transient potentially pathogenic microorganisms, outgrowth of indigenous potentially pathogenic microorganisms, and/or proliferation of antimicrobial resistant strains. The barrier effect may be disrupted by the action of any antimicrobial drug in the intestinal microflora.

If disruption of the colonization barrier is the endpoint of concern (i.e. the property of the flora that prevents the overgrowth of transient or indigenous, potentially pathogenic microorganisms (Vollard & Clasener, 1994), then either *in vitro* (e.g., continuous or semi-continuous culture systems) or *in vivo* test systems (e.g., human flora-associated rodent test systems) may be used to determine a NOEL for this endpoint. While these complex models of the human intestinal flora better approximate human intestinal flora, there is some recognition that standardized antimicrobial susceptibility testing of at least 100 strains of bacteria normally inhabiting the colon may be used to derive an ADI. MIC data may be used in absence of data from more complex model systems to estimate a conservative microbiological ADI even though MIC data indirectly assesses changes in bacterial populations. If the antibiotic concentration is below the levels that inhibit cell growth, it could be assumed that the bacteria responsible for the barrier effect would not be affected. Vice versa, if bacteria are not allowed to grow due to low concentrations of antimicrobials, an unbalanced flora could allow the establishment of a pathogen that is not sensitive to the antimicrobial in study. The ADI derived from MIC data is conservative because the inoculum density used for testing in order of magnitude is lower than the bacterial population of the colon. In addition, growth conditions in MIC testing (growth medium, pH, lack of fecal solids, lack of microbial interactions and drug metabolism, etc.) minimize the potential of drug inactivation. If MIC testing is used as an option to derive an ADI, the median MIC obtained by standard methods such as those of the National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 1993, NCCLS, 1994) of the most appropriate genus should be used to determine a NOEL. It

is recommended that at least 10 isolates from each of the most representative bacteria be obtained from healthy human volunteers. A summary of the ADIs using a microbiological endpoint determined at JECFA meetings is shown in Table 3.

F. Assessing the risk of exposure to antimicrobial residues

Microbiologically active residue of veterinary antimicrobials in food may in some way contribute to antibiotic resistance either by modifying the bacterial flora in the human gut or by selecting for resistant bacteria in the meat which can then be transferred to humans in food. This could lead to treatment failure if the antimicrobial or its drug class is routinely used for treatment of infectious disease in humans. It should be noted that antimicrobial residues in food are a different issue from resistant organisms acquired from food or other persons but potentially related to the issue of antimicrobial selection pressure that could play a role in transmission and carriage of resistant intestinal microfloral species to and among humans. Antimicrobial residues in foods makeup a small fraction of total antimicrobials to which persons are exposed to in terms of either frequency or dose that it seems unlikely they contribute significantly to resistance of intestinal microflora in humans. Furthermore, humans are never exposed continuously to antimicrobial residues and the food of animal origin will be diluted with the total mass of food and fluid ingested. The food commodities in which the residue is present may not be part of the daily diet of the consumer or may not be present in the edible portion of the commodity (Fitzpatrick, 1995). Moreover, since drug residues are so low due to the conservative nature of the assignment of ADIs, MRLs, withdrawal times, and since not all animals are treated nor slaughtered at legally established withdrawal times, it is understandable that there have been no reported instances in which adverse reactions to humans have been documented. However, the failure to report an instance does not necessarily mean that no instances have occurred. Regulatory agencies require microbiological, toxicological and chemical residue studies as part of the safety evaluation of veterinary drugs to set ADIs, MRLs and drug withdrawal times to limit any risk of unnecessary exposure to a person ingesting the food commodity..

Gathering evidence to determine whether or not antibiotic residues in food can modify the antimicrobial resistance profile of human intestinal microflora is problematic (JETACAR, 1999). First, not all food-producing animals are treated with antimicrobials, and of these few will have tissue residues at the MRL. Second, *in vivo* adsorption, chemical or bio-inactivation via metabolism and dilution of antimicrobial residues in the human gut may further lower the concentration of antimicrobials in the lumen of the gastrointestinal tract that is available to come in contact with the intestinal microflora. Third, the degradation of residues associated with food processing and cooking may result in lower concentrations of microbiologically active residues in the prepared food. Distribution of bacterial flora in the human bowel in relation to these processes is another consideration. Therefore, dietary consumption of microbiologically active residue of veterinary antimicrobials is unlikely to be a major factor in the development of antimicrobial resistance in humans.

From a toxicological viewpoint, the safety evaluation and procedures to set ADI's, MRLs, and drug withdrawal times for antimicrobials are not expected to cause toxic reactions in target species or in humans as long as they are used at the correct dosage and at the levels permitted. Most antimicrobial residues, if present in food, would be at concentrations too low for toxic effects. The toxicological endpoint or microbiological endpoint resulting in the lowest ADI ultimately drives the overall ADI. This hazard analysis, coupled with exposure assessment based on robust residue and depletion analysis,

as well as conservative assumptions regarding potential ingestion rates by individuals helps to minimize the risks of exposure to any toxicological potential for the consumer.

G. Summary/conclusions/recommendations

The ingestion of residues of antimicrobial compounds in food of animal origin may pose a danger to human health by colonization barrier disruption leading to pathogenic bacteria overgrowth or by exerting a selective pressure on the intestinal microflora thus favoring the growth of microorganisms with natural or acquired resistance.

Studies have been developed which are used in regulatory submissions to determine adverse toxicological and microbiological effects of veterinary drug residues. An extensive literature review revealed no reported episodes of human health effects that occur as a result of antimicrobials present as residues in foods. However, the failure to find recorded adverse health effects does not necessarily mean that they have not occurred

A harmonized approach is necessary in evaluating veterinary antimicrobial drug residues in food based on their effects on intestinal microflora. Europe, the United States and international regulatory organizations have different approaches as outlined in Table 4. The VICH Safety Working Group is developing a unified approach in evaluating data to determine the impact of veterinary antimicrobial drug residues in food and human intestinal microflora. It is quite similar to the JECFA and FDA decision tree/pathway approach. This approach should be considered by national and international regulatory authorities and committees involved in the safety evaluation and risk assessment of chemicals in food to ensure consistency and transparency in determination of microbiological ADIs.

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Table 1. Methods for measuring the effects of antimicrobial compounds on the human intestinal microflora.^a

METHOD	ADVANTAGES	DISADVANTAGES†	Recommended use in testing for
<i>In vitro</i>			
Short term anaerobic incubation (1-4h) of fecal suspensions	<ul style="list-style-type: none"> - Rapid method to screen fecal specimens for immediate drug impacts on bacterial metabolism, bacterial numbers, resistant (insensitive) organisms - Bacterial flora representative of original fecal inoculum - Relatively inexpensive, simple to perform - No ethical restrictions 	<ul style="list-style-type: none"> - Does not address long term exposure effects on colonization barrier effects and resistance emergence - Does not take into account host metabolism - Models fecal flora , not necessarily intestinal flora - The impact of variability of fecal inoculum on outcome has not been examined. 	<ul style="list-style-type: none"> - Microbial metabolism of antimicrobials - Fecal binding, chemical transformation and/or biotransformation to cause drug inactivation - Relatively fast changes in bacterial and drug resistant populations, colonization barrier as a result of short-term exposure of the drug - Impact of drug on bacterial functional endpoints, including hydrolytic and reductive enzyme reactions, gas production, volatile and non-volatile fatty acid formation
Pure culture determination of minimal inhibitory concentration (MIC)	<ul style="list-style-type: none"> - Relatively simple to screen a number of cultures representative of human gastrointestinal tract - Relatively inexpensive, simple to perform - No ethical restrictions 	<ul style="list-style-type: none"> - Does not take into account drug interactions with fecal solids - Does not fully model complexity of ecological interactions in the intestinal tract - Standardized MIC tests do not represent <i>in vivo</i> intestinal conditions (bacterial numbers, fecal solids, eH, pH, bacterial interactions, etc.) - Does not provide data to address resistance emergence - Models cultivable bacteria only - Does not simulate microflora or account for host metabolism - Does not address resistance emergence - No consensus on how to summarize MIC output data to estimate a no effect level in humans 	<ul style="list-style-type: none"> - Spectrum of drug activity - Determining an ADI using “JECFA formula” approach - Direct effects of low levels of antimicrobial agents on a specific bacterial species
Continuous and semi-continuous culture systems	<ul style="list-style-type: none"> - Samples are homogenous - Bacterial complexity of fecal inoculum retained, - Models microflora interactions in the human large intestine - Can examine long term, continuous or pulsed drug exposures - No ethical restrictions 	<ul style="list-style-type: none"> - Does not take into account host metabolism - Intestinal (physiological) conditions not represented - Does not take into account drug interactions with solids found in fecal specimens. - Representative of resistant organisms or resistance determinants not established. - Models fecal flora not necessarily intestinal flora - Impact of variability of fecal inoculum on outcome has not 	<ul style="list-style-type: none"> - Determining no effect concentrations for functional endpoints, including hydrolytic and reductive enzyme reactions, gas production, volatile and nonvolatile fatty acid formation, bacterial interactions, colonization barrier resistance emergence - Determining an ADI using “JECFA formula”

METHOD	ADVANTAGES	DISADVANTAGES†	Recommended use in testing for
		been examined. - Bacterial concentrations (10^9 cells/ml) are lower than in feces (10^{11} cells/g) - Resource intensive, technically difficult, most expensive among the <i>in vitro</i> test systems. - Few GLP laboratories available to conduct studies. - Studies needed to determine causes of variability in barrier effect studies before can be used for regulatory studies	- May be useful in determining potential for drug to be inactivated due to bacterial biotransformation.
Simulated gut models	- Straightforward/simple model - Models impact of food passage through stomach - Relatively inexpensive and simple to perform - Can screen a number of cultures representative of the human gastrointestinal tract - Models host digestion processes that may impact drug activity - No ethical restrictions	- Microbiological endpoint is survival of tested bacterial isolates, which can be overly simplistic - Does not fully model complexity of the gut system - Does not account for host metabolism - Intestinal conditions not represented (fecal solids, bacterial density, intestinal pH, bacterial interactions in the intestine) - Models cultivable bacteria only. - Does not address resistance emergence - Does not provide data to address resistance emergence - Models cultivable bacteria only. - Does not simulate microflora - No consensus how to summarize MIC output data to estimate a no effect level in humans.	- Determining an ADI using “JECFA formula” - May be useful to examine the potential for the drug to be inactivated.
Intestinal fed-batch culture	- Bacterial complexity retained - Can examine repeated, long-term or pulsed drug exposures to bacterial subcultures - Relatively inexpensive, allowing experimental designs examining more than one sources of fecal inoculation - No ethical restrictions	- May result in diminished complexity of flora with repeated transfers - Bacterial populations are periodically destabilized on cyclic basis, which may impact any colonization barrier studies - Does not take into account all aspects of host metabolism - Intestinal (physiological) conditions not represented - Does not take into account drug interactions with solids found in fecal specimens - No validation that resistance determinants in sub-cultures are representative of humans with repeated transfer - Impact of variability of fecal inoculum on outcome has not been examined. - Models fecal flora not necessarily intestinal flora - Bacterial concentrations (10^9 cells/ml) are lower than in feces (10^{11} cells/g)	- Functional endpoints, including hydrolytic and reductive enzyme reactions, gas production, volatile and nonvolatile fatty acid formation, bacterial interactions, colonization barrier effects, resistance emergence - Determining an ADI using “JECFA formula” - Potential for drug to be inactivated due to bacterial biotransformation.

METHOD	ADVANTAGES	DISADVANTAGES†	Recommended use in testing for
<i>In vivo</i>			
Conventional laboratory animals	<ul style="list-style-type: none"> - Relatively simple to perform; can control wide variety of dietary and environmental parameters. - Ability to monitor flora in different gut regions as well as the feces - Host metabolism occurs - Bacterial complexity is high - Least expensive among <i>in vivo</i> systems - Limited ethical considerations 	<ul style="list-style-type: none"> - Intestinal microflora differ among animal species - Human host interactions not included - High variability among individuals may occur - Coprophagy in certain species does not mimic eating patterns of humans - No validation that resistance determinants found in bacteria of GI tract of animals representative of those in humans - Open system: bacterial contamination during trial 	<ul style="list-style-type: none"> -Determining resistance emergence, colonization resistance and functional endpoints can be monitored - May be useful in determining potential for drug to be inactivated due to metabolism or transformation in intestine
Human flora associated rodents	<ul style="list-style-type: none"> - Relevant to humans, since the inoculant is fecal flora from humans - Can control variety of dietary and environmental parameters - Ability of monitoring the flora in different gut regions as well as the feces - Bacterial complexity is high - Host metabolism occurs - Limited ethical consideration 	<ul style="list-style-type: none"> - Expensive, germfree animal facilities required, - Human host interactions not included - Labor intensive - Coprophagy in certain species does not model human eating patterns - Introduction of gut flora which is different from conventional one, might affect physiology of human flora associated animals -Validation needed that resistance determinants in flora are representative of those in humans - Impact of fecal inoculum on outcome has not been examined. - Gut physiology (secretions, pH, peristalsis) of germfree animal may not be identical to that of humans - Models fecal flora, not necessarily intestinal flora 	<ul style="list-style-type: none"> - Determining resistance emergence, colonization barrier effects, and functional endpoints can be monitored - May be useful in determining potential for drug to be inactivated due to metabolism or transformation in intestine
Human volunteers	<ul style="list-style-type: none"> - Direct relevance to humans for safety evaluations - No interspecies extrapolation has to be made - Fecal samples can be obtained to determine microbial population changes and number of organisms resistant to antimicrobial 	<ul style="list-style-type: none"> - Ethical issue of using human subjects - Statistical significance due to human variability and high number of volunteers required to conduct study - Long monitoring periods - Most expensive system to use - Fecal flora may not be representative of colonic flora 	<ul style="list-style-type: none"> - Determining resistance emergence and colonization barrier effects. May be used for veterinary drugs that have approval for use in human medicine.

^aAdapted from (Corpet, 1992; Rumney and Rowland, 1992,1995;Nouws et al, 1994; CVMP, 1995; Silley and Watson, 1998; and Cerniglia and Kotarski, 1999)

†With the exception of MIC tests, none of these test systems have standardized protocols, or have been evaluated for test system variability.

Table 2. Decision-tree for determining adverse microbiological effects of residues of antimicrobial drugs in food producing animals (FAO/WHO, 2000)

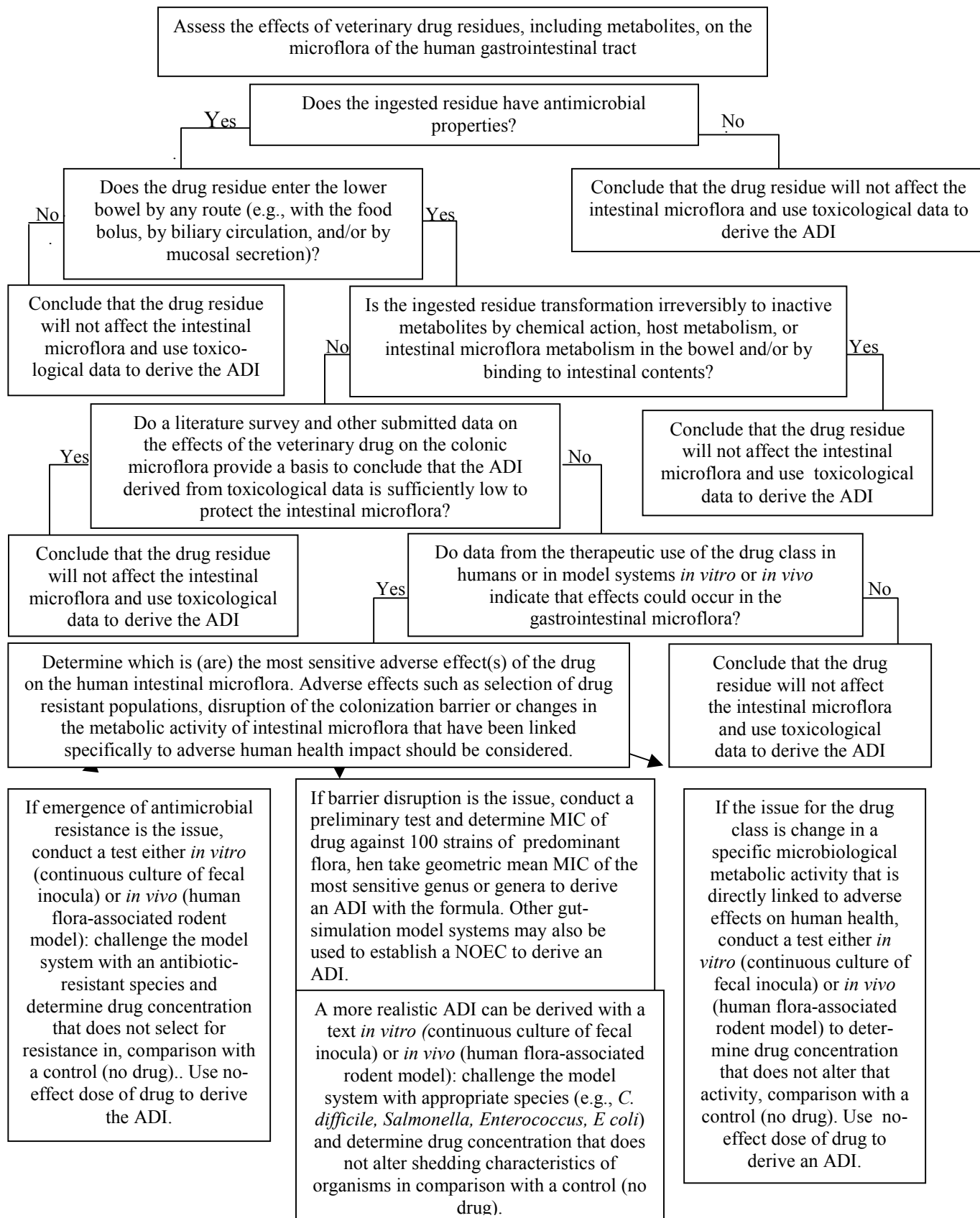


Table 3.

JECFA Published Evaluations of Microbiological ADI's of Antimicrobial Agents*			
		Microbiological ADI (micrograms/kg bodyweight)	REFERENCE
	Drug Class		
Ceftiofur	Cephalosporin	50	FAO/WHO (1996)
Cefuroxime	Cephalosporin	30	FAO/WHO (2002)
Danofloxacin	Fluoroquinolone	37	FAO/WHO (1997)
Dihydrostreptomycin and Streptomycin	Aminoglycoside	80	FAO/WHO (1995)
		120	FAO/WHO (1997)
Enrofloxacin	Fluoroquinolone	2	FAO/WHO (1997)
Flumequine	Quinolone	37	FAO/WHO (1997)
Gentamicin	Aminoglycoside	20	FAO/WHO (1998)
Lincomycin	Lincosamide	30	FAO/WHO (2000)
Neomycin	Aminoglycoside	160	FAO/WHO (1995)
Oxytetracycline, tetracycline, or chlortetracycline	Tetracycline	30	FAO/WHO (1998)
Sarafloxacin	Fluoroquinolone	0.3	FAO/WHO (1998)
Spectinomycin	Aminocyclitol	40	FAO/WHO (1994)
Spiramycin	Macrolide	50	FAO/WHO (1997)
Thiamphenicol	Chloramphenicol analog	5	FAO/WHO (2000)
Tilmicosin	Macrolide	40	FAO/WHO (1996)

*Not all published ADIs may be represented in this table.

Table 4. Conceptual Approaches for Determining a Microbiological ADI

	<i>JECFA</i>	<i>CVMP</i>	<i>FDA</i>	<i>VICH</i>
Reference	WHO Technical Report Series 893.	EMA/CVMP/234/01-Final Guideline	FDA/CVM Draft Guidance for Industry #52*	VICH Draft Guideline GL-36
Microbiological effects of concern	<ul style="list-style-type: none"> -Colonization barrier disruption -Emergence of resistance -Enzymatic activity directly linked to an adverse effect on human health 	<ul style="list-style-type: none"> -Reduction or elimination of barrier effect of normal intestinal flora -Development or increase pool of resistant strains through resistance transference from normal flora 	<ul style="list-style-type: none"> -Disrupt protective barrier effect provided by intestinal microflora colonization barrier -Emergence of resistance -Change in metabolic activity of microflora 	<ul style="list-style-type: none"> -Colonization barrier disruption -Increase in the populations of resistant bacteria
Conceptual approach	<ul style="list-style-type: none"> -“Comprehensive decision tree approach” to determine need for microbiological ADI -Determine if drug residues are active, enter, and remain in colon. If no, use ADI based on toxicological data. If yes, may use comprehensive literature survey to determine if drug residue will affect microflora at proposed toxicological ADI -If microbiological ADI must be determined, address most sensitive effect(s). -If concern is colonization barrier disruption, formula approach based on MIC data or other <i>in vitro</i> test may be used -If concern is barrier disruption, resistance emergence, and/or enzymatic activity, <i>in vitro</i> or <i>in vivo</i> testing may be used 	<ul style="list-style-type: none"> -A formula approach based on MIC data and correction factors are used to determine a microbiological ADI. Correction factors are assigned to take into account: 1) differences between <i>in vitro</i> and <i>in vivo</i> situations and 2) the potential of drug substance to cause selection and induction of resistance development. 	<ul style="list-style-type: none"> -“Pathway approach” to determine need for microbiological ADI. -Determine if drug residues are active, enter the colon, and remain in the colon. -If no, derive an ADI based on toxicological data. If yes, use available human data to determine adverse effect(s) of concern. -Use <i>in vitro</i> or <i>in vivo</i> testing to derive a microbiological ADI when concern is colonization barrier disruption and/or development of resistant bacteria and/or changes in microflora metabolism. 	<ul style="list-style-type: none"> -“Sequence of steps” to determine the need for a microbiological ADI. -Determine if drug residues are active, enter the colon, and remain in the colon. If no, use ADI based on toxicological data. If yes, determine if there is scientific justification to decide whether a microbiological ADI must be determined. If yes, a formula approach based on MIC data may be used when the concern is colonization barrier disruption -<i>In vitro</i> or <i>in vivo</i> testing may be used when concern is colonization barrier disruption and/or development of resistant bacteria

<p>Relevant data taken into account</p>	<ul style="list-style-type: none"> -Microbiological activity (MIC of relevant intestinal bacteria) -Availability of drug in colon (metabolism pharmacokinetics) -Activity of residue in the colon (fecal binding, inactivation, etc.) -Effect of drug on GI flora <i>in vitro</i> or <i>in vivo</i>, and assessment of toxicological ADI (changes in bacterial populations, colonization barrier, resistant bacterial populations, etc.) -Endpoint(s) of concern for class of drug if a microbiological ADI is needed -Human data with appropriate safety factor 	<ul style="list-style-type: none"> -Microbiological activity (MIC of relevant intestinal bacteria) -Availability of drug in colon (metabolism pharmacokinetics) -Activity of residues in the colon (fecal binding, inactivation, etc.) -Data to determine NOEL obtained in HFA rodents when induction of resistance and reduction of barrier effect are studied -<i>In vitro</i> MIC data 	<ul style="list-style-type: none"> - Microbiological activity (MIC of representatives of intestinal bacteria) - Availability of drug in colon (metabolism, pharmacokinetics) - Activity of residues in colon (fecal binding, inactivation, etc) - Endpoint(s) of concern for the class of drug if a microbiological ADI is needed (<i>in vitro</i> or <i>in vivo</i> preliminary or definitive studies) 	<ul style="list-style-type: none"> - Microbiological activity (MIC against relevant intestinal bacterial) - Availability of drug in colon (adsorption, distribution, metabolism) - Activity of residues in the colon (fecal binding, inactivation, etc.) - Scientific justification to eliminate need for testing one or both endpoints of concern (literature, case studies, etc.) - Endpoint(s) of concern for class of drug if microbiological ADI is needed
<p>Testing to establish an ADI for increase in population of resistant bacteria</p>	<p>-<i>In vitro</i> tests (continuous culture of fecal inocula) or <i>in vivo</i> test (HFA-rodents, mice, rats, pigs). Challenge models with resistant strains and determines drug concentrations that a) does not select for resistance) or b) does not select for challenge bacteria (NOEL).</p>	<p>-MIC tests based on recognized guidelines (e.g., NCCLS), using different inoculum concentrations.</p>	<p>-<i>In vitro</i> or <i>in vivo</i> studies in model systems (as those recommended for colonization barrier disruption) for determining a NOEL.</p>	<p>-<i>In vitro</i> test systems: continuous, semi-continuous, and fed-batch cultures may be useful to evaluate long-term exposure and obtain a NOEC. <i>In vivo</i> test systems: HFA-rodents, conventional animals. Determination of a NOEL. Further studies needed for validation of <i>in vitro</i> and <i>in vivo</i> test systems.</p>
<p>*This draft guidance may be further revised at a later date in accordance with recommendations from the VICH guidance development, concerning proper tests, model systems and protocols.</p>				

Chapter 8 Economical impacts of non-human antimicrobial usage and antimicrobial resistance, including impacts of intervention measures

As discussed in the introduction, non-human antimicrobials are used for therapy, disease prophylaxis and growth promotion. Potential economic benefits derive from improved animal health and production efficiency. Potential costs include drug costs, antimicrobial resistance-related health costs in animals and humans, and losses in domestic or international markets because of diminished consumer confidence. This chapter provides an overview of the available scientific information pertaining to these subjects.

A. Economic benefits of non-human antimicrobial use

International and national reports emphasize that antimicrobials are vital medicines for treatment of bacterial infections (5,7,13,14) and therefore enhance animal health and welfare. Some of these reports provide specific examples of important animal diseases that are treated or prevented with antimicrobials (5,7), however attempts were not made to estimate economic benefits from therapeutic use. There is an extensive literature, not reviewed here, documenting the therapeutic efficacy of various antimicrobials in animals, and in some cases, costs of alternative treatment strategies for specific disease conditions.

Economic assessments have instead tended to focus on antimicrobial growth promoters because their continued use is challenged. Various experimental and field studies have shown that under certain conditions antimicrobial growth promoters may enhance production by 1-11% (5,7,9,11) by enhanced feed efficiency or other effects. It is not the purpose of this report to extensively review the possible mechanisms of action of antimicrobial growth promoters, but it is reported that a proportion of the beneficial effect of these drugs is mediated through suppression of pathogens and disease; the benefits are reduced or eliminated in pathogen-free animals and are most apparent in animals raised under conditions of poor hygiene (5,7). It has also been reported that benefits are less pronounced in recent times because of improvements in animal management and husbandry, improved vaccines and improved genetics (5). An important motive for antimicrobial growth promoter use in poultry is prevention of necrotic enteritis, a bacterial infection caused by *Clostridium perfringens* overgrowth secondary to intestinal damage by coccidia infection (*Eimeria* spp.). In pigs, antimicrobial growth promoters have been used for prophylaxis of diarrhea (e.g. olaquinox for *Lawsonia intracellularis* infection in weaned pigs) (5,7). In feedlot cattle, antimicrobial growth promoters are sometimes used to prevent liver abscesses secondary to ruminal acidosis, and ionophore antimicrobials (e.g. monensin) are widely used in some countries to enhance feed efficiency (7). Economic effects of antimicrobial growth promoters are further discussed below.

It has been suggested that use of antimicrobials in animals may benefit human health by treatment of zoonotic infections in animals (7). While it has been shown that antimicrobial therapy is effective in treatment of some clinical zoonotic infections of animals, for example salmonellosis in pigs or calves, there is little published evidence that such treatment has reduced the overall load of foodborne pathogens in animals or food, or the incidence of human infection. According to a recent literature review commissioned by the U.S. Food and Drug Administration (FDA) most relevant studies do not show an effect of antimicrobial use in food animals on pathogen load, although the literature was deemed sufficient only for *Salmonella*, and was limited to swine and poultry (3).

B. Economic costs of non-human antimicrobial use

Direct costs are largely confined to the costs of the drugs themselves (not reviewed here). Indirect costs include costs related to antimicrobial resistance in human and animal pathogens. The Codex Committee on Food Hygiene noted that economic consequences of infections with antimicrobial resistant pathogens may relate to various factors, including patients' incapacity to work, hospital costs, increased medication and other treatment costs, and increased costs in the food industry (2).

Australia's JETACAR stated that definitive studies on the economic impacts of resistance in humans are scant, and noted that antimicrobials needed to treat resistant infections tend to be more expensive than those used for susceptible infections, that some need to be administered intravenously, which may require hospitalization, acquisition of resistant bacteria may prolong hospital stay, and therapy that has failed because of resistance may increase costs because an additional antimicrobial is needed and there may be additional laboratory tests required (5). These costs apply to resistant infections in general, and not just those originating in animals.

Problems with antimicrobial resistance have been reported in some, but not all pathogenic bacteria of animals (see (1,7,8,11) for additional information), and the prevalence of resistance varies considerably in different countries. Resistance in animal pathogens is an economic burden for some of the same reasons stated above for resistant human infections. In addition to multi-drug resistant strains of *Salmonella*, which can cause illness in animals and humans, resistance problems have been reported in a variety of animal pathogens, for example, *Escherichia coli* of poultry and swine, *Pasteurella multocida* and *Mannheimia haemolytica* of cattle, *Pasteurella* of turkeys, *Actinobacillus pleuropneumoniae* of swine, *Staphylococcus aureus* of dairy cattle, *Aeromonas salmonicida* ssp. *Salmonicida* of salmonid fish and *Clostridium perfringens* of poultry (1,7,8,11).

There is little published evidence that non-human antimicrobial use or resistance have major effects on domestic or international trade. Lauritsen, 2002 (6) reported that in Denmark, these factors ranked below price and quality in the marketing of pork. Concerns have also been expressed that the issue of antimicrobial resistance could be used as a basis for international trade restrictions (1).

C. Economic effects of interventions

The U.S. National Academy of Sciences published a report in 1999 (7) that assessed the economic impacts from banning use of subtherapeutic antimicrobials (collectively including drug claims for feed efficiency, growth promotion and in some cases, disease prophylaxis) in U.S. agriculture. The report stated that nearly 100% of chickens and turkeys, 90% of swine and veal calves, and 60% of beef cattle in the U.S. were fed rations medicated with antimicrobials. Based on assumed improvements in feed efficiency of 2.7-6.5 %, depending on species of livestock, the estimated cost of a ban on subtherapeutic drug use was \$1.2-2.5 billion overall, or \$4.84 to \$9.72 per year on a per capita basis.

In 2001, Hayes et al (4) estimated the economic impact of a ban on feed-grade antimicrobials on U.S. pork production and consumer prices of pork. Based on data from the Swedish and Danish pork industries and assumptions about likely costs (including capital costs associated with additional space and feed troughs) under U.S. conditions, they estimated that a U.S. ban would most likely increase the

costs of production by \$6.05 per animal initially, eventually dropping to \$5.24 per animal after 10 years. Their analyses predicted higher pork prices for consumers of \$0.05 per pound.

Considerable attention has been given to economic and animal health effects of changes in antimicrobial growth promoter use policy in Europe, particularly in Sweden and Denmark. In 2003, the World Health Organization (WHO) published an expert panel review of Denmark's program for termination of the use of antimicrobial growth promoters in food animal production, particularly swine and poultry (13). The review was comprehensive and was made possible by the extensive national surveillance and animal production data that were collected in Denmark before, during, and after the period of termination. Surveillance and monitoring data were supplemented by many targeted studies that were carried out by Danish scientists. Using this information, the panel examined consequences of the program to human health, animal health and welfare, environmental impact, animal production, and national economy. Effects on animal health and welfare, food safety, animal production and national economy are relevant to this chapter.

Following termination of antimicrobial growth promoters, there was a significant increase in diarrhea among pigs in the post-weaning period, and a less pronounced and transient increase in diarrhea in finishers. In broilers, there were no major health problems attributable to termination. A minor increase in necrotic enteritis incidence in poultry was observed, but this may have been mitigated by continued use of ionophores for prophylaxis of necrotic enteritis and coccidiosis. No additional animal welfare impacts were identified.

In weaner pigs there was some loss of productivity (2.6% reduction in growth rate and 0.6% increase in mortality) following termination of antimicrobial growth promoters. Finisher pig productivity was not noticeably affected. Effects on poultry productivity were small (0.9% reduction in feed efficiency) and offset by savings in the cost of antimicrobial growth promoters.

Termination of antimicrobial growth promoters did not appear to affect the incidence of antimicrobial residues in foods or the incidence of human *Salmonella*, *Campylobacter*, or *Yersinia* infections in humans; the major zoonoses in Denmark associated with consumption of pork and poultry.

An analysis of the economic impact of termination on the Danish economy showed that the net costs to producers was estimated at 7.75 DKK (1.04 €) per pig produced and no net cost for poultry. An economic model of the Danish economy predicted that as a result of antimicrobial growth promoter termination, pig production would be about 1.4% per annum lower and poultry production 0.4% per annum higher (because of reduced feed costs) than expected. The model estimated that the impact on the Danish economy would be a reduction of 0.03% (363 million DKK (48 million €) by 2010 at 1995 prices) in real Gross Domestic Product (GDP).

The panel concluded that the negative effects of antimicrobial growth promoter termination were mainly attributable to their disease prophylaxis (i.e. disease prevention) properties. Furthermore, it also concluded that "Overall, the likely impact of withdrawal of antimicrobial growth promoters on pig and broiler production, and for the Danish economy, is a relatively small but negative effect. Some of these costs (e.g. increased therapeutic antimicrobials, reduced growth rate) have been measured and were not large, but others, especially some costs associated with modifications of the production systems, are difficult to measure and were not included in this report, although they may have been substantial for

some producers. The small negative effect may be, at least partially, offset by the benefits of increased consumer confidence in, and demand for, Danish pig and poultry meat produced without antimicrobial growth promoters.”

In the panel’s judgement, Denmark’s experiences should be generally applicable to other countries with similar animal production conditions (e.g. intensive, with closed housing, good biosecurity and relatively high health status).

D. Analysis: economic impacts

There is general agreement that therapeutic antimicrobials are very beneficial for the treatment of active bacterial infections of animals. Little interest has been shown in measuring the economic impact on society of non-human therapeutic treatments, or considering the costs of not allowing such treatments, perhaps because these are considered essential for animal welfare reasons. In contrast, there continues to be considerable debate about the usefulness and economic impact of antimicrobial growth promoters. While there is evidence from research studies that certain growth promoters (e.g. ionophores) improve feed efficiency or otherwise promote growth in some species of livestock (e.g. feedlot cattle), there is increasing evidence that much of the impact of most antimicrobial growth promoters is mediated through disease prophylaxis. Therefore, for many growth promoters, further justification of use appears to be based on animal health and welfare reasons rather than production efficiency. This has important implications to resistance risk management.

There is little documented evidence that non-human antimicrobial use decreases the burden of illness in humans due to antimicrobial-susceptible foodborne zoonotic infections, and therefore the costs of these diseases. Conversely, to the extent that non-human antimicrobial uses contribute to the burden of human illness from antimicrobial resistant infections (the subjects of chapters 5&6), such uses will increase health care costs. Future costing studies in this area should account for possible effects on resistance from use of drugs selected because they require a reduced milk or meat withdrawal period. For example, by using a third generation cephalosporin the farmer may benefit if no milk must be discarded after treatment of a dairy cow, however this drug is probably more important in human medicine than another drug for which milk must be withdrawn for a period to prevent residues. Antimicrobial resistance is also costly to animal health, although estimates of these costs have not been made. Resistance problems have been reported in several important pathogens of animals. Such resistance can lead to production losses, and increased costs associated with use of more expensive antimicrobials than would otherwise be used, and ancillary diagnostic testing.

A few quantitative economic assessments of antimicrobial growth promoter use have been conducted. In general, these were based on assumptions that do not clearly distinguish disease prophylaxis effects from feed efficiency or other effects. There is a need for further analyses that more clearly identify benefits and costs in these two areas. The recent WHO panel review of Denmark’s program for termination of the use of antimicrobial growth promoters in food animal production (12) is to date the most comprehensive and detailed evaluation of the economic effects of non-human antimicrobial use.

E. Summary/conclusions/recommendations

The societal benefits of therapeutic antimicrobial use in animals are assumed and unchallenged and have not been subjected to economic analysis. In contrast, the economic benefits of antimicrobial growth promoters have been estimated and are debated and uncertain. Some estimates, based on targeted studies, suggest that the animal production benefits are in the order of 1-11%, while other evidence, notably the review of Denmark's program of antimicrobial growth promoter termination in swine and poultry production, suggests that the production gains are considerably less. There is growing evidence that many antimicrobial growth promoters derive much of their benefit from disease prophylaxis, rather than enhanced feed efficiency or other effect. To facilitate resistance risk management, future economic analyses of non-human antimicrobial use should attempt to segregate benefits and costs in terms of feed efficiency, disease prophylaxis and therapy on a drug and animal species-specific basis.

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