

## Workshop report: The 2012 Antimicrobial Agents in Veterinary Medicine: exploring the consequences of antimicrobial drug use: a 3-D approach<sup>1</sup>

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Antimicrobial resistance is a global challenge that impacts both human and veterinary health care. The resilience of microbes is reflected in their ability to adapt and survive in spite of our best efforts to constrain their infectious capabilities. As science advances, many of the mechanisms for microbial survival and resistance element transfer have been identified. During the 2012 meeting of Antimicrobial Agents in Veterinary Medicine (AAVM), experts provided insights on such issues as use vs. resistance, the available tools for supporting appropriate drug use, the importance of meeting the therapeutic needs within the domestic animal health care, and the requirements associated with food safety and food security. This report aims to provide a summary of the presentations and discussions occurring during the 2012 AAVM with the goal of stimulating future discussions and enhancing the opportunity to establish creative and sustainable solutions that will guarantee the availability of an effective therapeutic arsenal for veterinary species.

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<sup>1</sup>To view the meeting agenda and to obtain a biographical summary of the keynote speakers and moderators, please go to <http://www.aavmconferences.com/2012/>

### INTRODUCTION

The threat of infectious diseases is a concern impacting both human and animal health. The emergence of antimicrobial resistance threatens the effectiveness of our existing therapeutic arsenal. Therefore, the development and spread of pathogenic and nonpathogenic organisms that carry resistance genes are

a cause of increasing concern (Anon 1, Anon 8). However, this is not a new phenomenon, and there is evidence that antimicrobial resistance is an ancient mechanism in the life cycle of bacteria (D'Costa *et al.*, 2011; Bhullar *et al.*, 2012).

As stated by Vaarten at the 2012 meeting of the World Organization for Animal Health (OIE), antimicrobial use in animals is an essential part of our efforts to control those bacterial

infections responsible for causing pain and suffering, tissue damage, organ dysfunction, weight loss, and loss of productivity. The benefits associated with judicious drug use include protecting animal health and welfare as well as supporting the availability of foods of animal origin. It also reduces the risk of spreading (zoonotic) infections, allows for the containment of potentially large-scale epidemics, and supports the protection of people's livelihoods and animal resources (Simmons, 2011).

The 2012 meeting of Antimicrobial Agents in Veterinary Medicine (AAVM) was convened to promote a dialog on creative and sustainable solutions to the global threat of antimicrobial resistance. Emphasizing the role of 'SCIENCE' as the mechanism for enhancing our understanding of the problem at hand, the theme of a 3-D approach was selected because of the inextricable interactions between humans, the treated animals, the environment, and wildlife. Through the platform of invited lectures and subsequent discussions, the 2012 AAVM provided an opportunity for research scientists, regulatory scientists, veterinary practitioners, and drug sponsors to explore practical solutions for important public health challenges.

## WORKSHOP SUMMARY

### *Part 1: Regulatory approach to address veterinary antimicrobial use concerns*

*Critically important drugs for human health: a WHO/FAO/OIE perspective.* The first Joint Food and Agriculture Organization (FAO)/OIE/World Health Organization (WHO) Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific assessment took place in Geneva in 2003 <http://www.who.int/foodsafety/publications/micro/en/amr.pdf>. A Second Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance:

Management options was held in 2004 in Oslo, Norway [http://www.oie.int/fileadmin/Home/eng/Conferences\\_Events/docs/pdf/WHO-CDS-CPE-ZFK-2004.8.pdf](http://www.oie.int/fileadmin/Home/eng/Conferences_Events/docs/pdf/WHO-CDS-CPE-ZFK-2004.8.pdf).

Thereafter, the WHO convened an Expert Meeting on Critically Important Antimicrobials for Human Medicine, in Copenhagen, Denmark, in May 2007 (Anon 7) and a Joint FAO/WHO/OIE Expert Meeting on Critically Important Antimicrobials in November 2007 (Anon 8). The joint FAO/WHO/OIE report provided a list of critically important antimicrobials in humans and in terrestrial food-producing animals. This list was further modified in 2011 by the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR). This revised list included not only newly developed drugs and scientific information but also a list of the specific compounds within each of these classes according to their use within either human or veterinary medicine (Anon 7). The resulting AGISAR ranking of drug classes in accordance to their importance to human medicine is provided in Table 1.

Much overlap is seen between compounds of importance to human and to animal health. Therefore, it was recommended that those drug classes listed as the highest for risk management strategies should include the (fluoro)quinolones, 3rd and 4th generation cephalosporins, and the macrolides. Resistance against these drug groups is detected in foodborne pathogens, namely *Salmonella* spp. and *Campylobacter* spp., and the commensal *Escherichia coli*.

It is important to emphasize that while these discussions focused solely on antimicrobial use in food-producing animals, it was concluded that antimicrobial use in nonfood-producing animals should also be subject to the prudent use provisions of the OIE Terrestrial Animal Health Code, 2012 (Anon 2).

*United States Food and Drug Administration approach for addressing veterinary antimicrobial use concerns.* To address

**Table 1.** Categorization of antimicrobials used in human medicine according to their importance in treatment of disease

Critically important antimicrobials	Highly important antimicrobials	Important antimicrobials
Aminoglycosides*	Aminopenicillins	Aminocyclitols
Carbapenems and other penems	Amphenicols*	Cyclic polypeptides
Cephalosporins (3rd and 4th generations)*	Cephalosporins (1st and 2nd generations) and cephamycins*	Nitrofurantoin*
Cyclic esters	Lincosamides*	Nitroimidazoles
Fluoro and other quinolones*	Penicillins (Antistaphylococcal)*	
Glycopeptides*	Pleuromutilins*	
Glycylcyclines	Pseudomonic acids	
Lipopeptides	Riminofenazines	
Macrolides and ketolides*	Steroid antibacterials	
Monobactams	Streptogramins*	
Oxazolidinones	Sulfonamides, dihydrofolate reductase inhibitors, and combinations*	
Penicillins (natural, aminopenicillins, and antipseudomonal)*	Sulfones	
Polymyxins	Tetracyclines*	
Rifamycins		
Drugs used solely to treat tuberculosis or other mycobacterial diseases		

\*Member(s) in the classes may have veterinary uses. They are examples and variations may exist among various regions or countries.

public health concerns associated with the use of antimicrobials in food-producing animals, the United States Food and Drug Administration (US FDA) Center for Veterinary Medicine (CVM) published three regulatory documents: (i) Guidance for Industry (GFI) #209 (Judicious use of Medically Important Antimicrobial Drugs in Food-Producing Animals; Anon 4); (ii) draft GFI 213 (New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209; Anon 5); and (iii) the Veterinary Feed Directive (VFD; Anon 3).

- a) GFI #209: This guidance is built on the principle that 'use of medically important antimicrobial drugs in food-producing animals should be limited to those uses that are considered necessary for assuring animal health'. Furthermore, the administration of medically important antimicrobial drugs in food-producing animals should be limited to uses that include veterinary oversight or consultation.
- b) Draft GFI #213: This document describes implementation of the key principles in GFI #209 based upon concepts set out in GFI #152, 'Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to their Microbiological Effects on Bacteria of Human Health Concern', which includes an appendix that ranks antimicrobial drugs as 'critically important', 'highly important', or 'important', in regard to their relevance to human medicine. This draft GFI provides a process for updating product labels and describes the data requirements for adding new indications. In this regard, new prevention uses are expected to include a defined dose duration, an effective therapeutic dose level, the disease at-risk population, and mechanisms for veterinary oversight (i.e., changing marketing status from over the counter [OTC] to either prescription or the VFD).
- c) The VFD: This provides a mechanism for requiring producers to secure approval from a veterinarian prior to the administration of medicated articles.

The VFD is the primary mechanism for veterinary oversight of antimicrobials administered in animal feed. It is prescription-like, but is intended solely for feed. It serves as an alternative to prescription status for certain therapeutic animal pharmaceuticals for use in feed. In essence, a VFD is a mechanism requiring a producer to get approval from a veterinarian for antibiotics used in feed. The Federal Food Drug and Cosmetic Act (FFD&C) requires that medicated feeds needing veterinary oversight be designated as VFDs. Regulations on the use of VFDs were finalized in January 2001. The FDA intends to move all medically important antibiotics out of OTC status to VFD status. Expressed concerns with the VFD include as follows:

- a) The potential for prescription status requirements would lead to major disruptions of existing marketplace practices for drug sponsors, feed manufacturers, and animal producers.

- b) State pharmacy laws and regulations intended to apply only to the dispensing of other dosage forms of drugs, not to medicated animal feed.
- c) Statutory limitations on labeling and marketing practices that would place covered drugs and feeds at a commercial disadvantage when compared with OTC-medicated feeds.

Currently, there are only 2 VFD drugs, tilmicosin and florfenicol.

There are several ongoing programs that facilitate efforts to address the challenge of antimicrobial resistance. These include the US Department of Agriculture (USDA), Antimicrobial Resistance Work Plan involving the Food Safety Inspection Service, Agricultural Research Service and Animal and Plant Health Inspection Service, and the National Antimicrobial Monitoring System (NARMS), which was jointly established by the FDA, USDA, and the US Centers for Disease Control and Prevention for the purpose of monitoring resistance among foodborne pathogens from humans, retail meats, and animals <http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm>.

In the 112th US Congress, the leading bill on antimicrobial resistance was the *Preservation of Antibiotics for Medical Treatment Act of 2011*. As described by the Congressional Research Service, which is a nonpartisan division of the Library of Congress, this Act was intended to amend the FFD&C Act to require the Secretary of Health and Human Services to deny an application for a new animal drug that is a critical antimicrobial animal drug unless the applicant demonstrates that there is a reasonable certainty of no harm to human health due to the development of antimicrobial resistance attributable to the nontherapeutic use of the drug. This amendment defines 'critical antimicrobial animal drug' as a drug intended for use in food-producing animals that contains specified antibiotics or other drugs used in humans to treat or prevent disease or infection caused by micro-organisms. The bill was intended to require the Secretary to withdraw approval of a nontherapeutic use of such drugs in food-producing animals 2 years after the date of enactment of this Act unless certain safety requirements are met. This bill was introduced on March 9, 2011, in a previous session of Congress, but was not enacted. (<http://www.govtrack.us/congress/bills/112/hr965>).

*The European Union approach to stimulate the prudent use of antimicrobials in animals.* Several landmark regulatory actions have had a significant influence on the use of antimicrobial agents on European farms. These include (Cogliani *et al.*, 2011):

- a) 1969: SWANN Report: Agricultural use of antibiotics (<http://hansard.millbanksystems.com/commons/1969/nov/20/use-of-antibiotics-in-animal-husbandry>). This report requires the ban of certain antibiotics (e.g., penicillin and tetracyclins for nonmedical (growth-promoting purposes) and recommends placing all antibiotics under veterinary prescription.

- b) 1986: Sweden bans the use of antimicrobials for growth-promotion in agriculture, as requested by Federation of Swedish Farmers.
- c) 1997: the European Union bans the use of avoparcin as a growth promoter due to its potential cross-resistance with vancomycin.
- d) 1999: The Steering Committee of the European Commission recommends phasing out the use of medically important antimicrobials as growth promoters and implements disease prevention methods. The ban included olaquinox and carbadox. Authorization was suspended on bacitracin, tylosin, spiramycin, and virginiamycin. [http://ec.europa.eu/food/fs/sc/ssc/out50\\_en.pdf](http://ec.europa.eu/food/fs/sc/ssc/out50_en.pdf)
- e) 2006: The European Commission issued a total ban of all antimicrobial growth promoters, including flavomycin/bambermycin and Zn-bacitracin.

Since 2006, all veterinary use of antimicrobials requires a prescription (POM status, prescription-only medicines), and that all licensed veterinary products are prescription-only drugs. In addition, screening for methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum  $\beta$ -lactamase (ESBL)-producing *E. coli* in animal populations was initiated. Along with these actions, hospital-acquired MRSA in companion animals was revisited, and a memorandum was issued against the use of the 3rd and 4th generation cephalosporins. The registration of antimicrobial use on farms was initiated. Some countries, such as Denmark and the Netherlands, have established action levels for the frequency of treatments (expressed as daily doses per animal per year).

While various applications for antibiotics in farm animal practice are restricted and controlled, research activities to improve the range of vaccination programs are encouraged and supported. This applies also to the use of feed additives that improve animal sustainability, including several classes of prebiotics and probiotics, organic acids, polysaturated fatty acids, enzymes, and other feed (nonantibiotic) ingredients. These nonantimicrobial compounds may help to stimulate the animal's immune system, reduce the risk for outbreak of infections, and increase feed utilization. Legal requirements have been set for the licensing of such compounds (EC 1831/2003), demanding quality assurance procedures, and a demonstration of activity and safety which is comparable to established drug-licensing procedures. The overall objective is disease prevention as a measure to reduce the need for therapeutic intervention.

## Part 2: Methodology and interpretation of antimicrobial testing

*Interpreting results from antimicrobial susceptibility tests.* Bacterial drug susceptibility is generally expressed relative to the minimum drug concentration that inhibits growth under a given set of *in vitro* test conditions (the minimum inhibitory concentration [MIC]). A prerequisite for any MIC determination is that it is generated through the use of quality-controlled methods.

MICs are estimated on the basis of serial dilutions. Drug potency is measured by the lowest concentration of an antimicrobial that inhibits bacterial growth after overnight exposure when the test tube contains an inoculum size of  $10^5$  colony-forming units (CFU) per mL of medium. The MIC serves as a benchmark against which changes in susceptibility can be tracked. It is also used to gauge dosages needed to treat infections based upon the application of PK and PD principles.

Despite reliance on these values, there are numerous potential shortcomings that need to be considered:

- a) **MIC:** The MIC reflects a single component of the interaction between a drug and a pathogen. The MIC value neither reflects the impact of very low (subinhibitory) concentrations on bacterial virulence nor can it distinguish between static or cidal effects on a pathogen. The mode of action for subinhibitory effects may not be the same as that associated with the MIC (e.g., Garch *et al.*, 2010). While nutrients or their precursors necessary for bacterial survival may be readily available in culture media, if absent *in vivo*, it can reduce pathogen viability and virulence. In this way, there can be differences in the *in vitro* vs. *in vivo* growth rates. In turn, this can lead to difference in the *in vivo* vs. *in vitro* concentrations needed to minimize bacterial growth.
- b) **Inoculum effects:** Although *in vitro* tests are conducted at an inoculum size of  $10^5$  CFU/mL, the microbial population in infected tissues may be as high as  $10^{10}$ – $10^{12}$  (Frisch *et al.*, 1942). For many compounds, the MIC increases as inoculum increases. Examples of drugs associated with an *in vitro* inoculum effect for *S. aureus* include ciprofloxacin, oxacillin, daptomycin, and vancomycin (the latter two potentially being associated with drug degradation), gentamicin and linezolid (whose decrease in drug efficacy with increasing inoculum may simply be attributable to a decrease in per-cell antibiotic concentration; Udekwu *et al.*, 2009). Conversely, upon examining the potential inoculum effect when *E. coli* is exposed to antibiotics that target the ribosome, Tan *et al.* (2012) observed that while aminoglycosides (e.g., streptomycin, puromycin, gentamicin, tobramycin, neomycin, and kanamycin) exhibit a decrease in activity, other antibiotics such as chloramphenicol or the tetracyclines are not associated with inoculum effects.
- c) **Sub-MIC effects:** The mode of action for subinhibitory effects may not be the same mode of action as that associated with the MIC (e.g., Garch *et al.*, 2010). See section 2.2.2 for further discussion.

*Establishment of appropriate dosage regimes: PK/PD considerations. General principles.* Within the framework of veterinary medicine, the therapeutic goals include treatment (when the patient has an active infection), control/metaphylaxis (e.g., when there is evidence of an outbreak), and prevention/prophylaxis (when there is a risk of a disease outbreak but no evidence of infection within the herd, farm, or house). Because of differences in the bacterial load (bioburden) and in the patient's physiological status, the concentrations

needed to achieve the desired therapeutic outcome can differ as a function of indication. This aspect is often incompletely addressed in the authorized dosage regimens. However, when administering antimicrobials to food-producing animals, any alteration of the dosage regime has also to consider the potential impact of such a dosage regimes on withdrawal periods and (microbial) human food safety.

Appropriate dosage regimens are a function of the exposure–response relationship associated with the action of the antimicrobial agent and the targeted therapeutic outcome (e.g., static effects, cidal effects, 1-log kill, and 3-log kill). Concentrations are assessed relative to free (unbound) plasma or serum drug concentrations because these are the concentrations most closely correlated with active drug concentrations at the site of action. Antimicrobial exposure–response (PK/PD) relationships are generally classified as being either time or concentration-dependent (Craig, 2007), with all drugs exhibiting components of both variables (Martinez *et al.*, 2012). PK/PD relationships are further expressed as time above MIC ( $T > MIC$ ), peak divided by the MIC ( $C_{max}/MIC$ ), or the extent of exposure (e.g., over a 24-h dosing interval) above the MIC (AUC/MIC). Examples of drugs falling within these classifications are as follow:

- a)  $T > MIC$ : penicillins, cephalosporins, monobactams, and carbapenems;
- b)  $C_{max}/MIC$ : aminoglycosides, fluoroquinolones, glycopeptides, and daptomycin;
- c) AUC/MIC: aminoglycosides, fluoroquinolones, glycopeptides, daptomycin, oxazolidinones, and tetracyclines. Although generally associated with time-dependent effects, the PK/PD parameter correlated with clinical outcome for most of the newer macrolides is also AUC/MIC due to its prolonged postantibiotic effect.

Examples of the numerical values of these indices that have been correlated with therapeutic success in human patients were summarized by Ambrose *et al.* (2007). The many variables contributing to a therapeutic response include (Martinez *et al.*, 2013):

- a) The susceptibility of the pathogen to the antimicrobial (i.e., the MIC of the antimicrobial agent).
- b) The characteristics of the pathogen response to the antimicrobial (e.g., cidal vs. static effects, and kill rate).
- c) The characteristics of the host–pathogen interaction and the ability of the drug to influence this interaction such as:
  - i) the ability of the pathogen to invade or colonize on host tissues;
  - ii) the secretion/release of toxins by the pathogen;
  - iii) the ability of subinhibitory concentrations to affect the pathogen and pathogen–host interactions; and the accumulation of certain antimicrobials in leukocytes.
- d) The PK of the drug and the dosage form.
- e) The dosage regime.
- f) The effect of the antimicrobial on the host disease response (e.g., drug anti-inflammatory effects).

- g) The host's immune status.
- h) The magnitude of the bacterial burden (bioburden) at the site of the infection.
- i) The propensity of the pathogen to form resistant genotypes and phenotypes (e.g., bacteria in biofilms).

Bioburden will likely differ when a drug is used for metaphylaxis, prophylaxis, or treatment. To ascertain whether or not bioburden can influence clinical and bacteriological outcomes, Ferran *et al.* (2011) used a non-neutropenic mouse-lung model of *Pasturella multocida* infection (Ferran *et al.*, 2011). In this study, clinical and bacteriological outcomes for infected mice were shown to be better, and the selection of resistance less frequent, when marbofloxacin was administered as an early intervention (10 h after inoculum administration) as compared to treatment at 32 h after inoculum administration. Moreover, the early dose of 1 mg/kg led to better clinical and similar bacteriological (eradication and selection of resistance) outcomes as compared to the later dose of 40 mg/kg marbofloxacin (curative therapy). These results support arguments favoring the utility of drug metaphylaxis from a clinical perspective, allowing for a reduction in both the total amount of drug administered per animal and the risk of selecting for pathogen resistance. Similar results have been obtained with amoxicillin and cefquinome in the same model.

*Estimating the dose.* When dealing with drugs associated with concentration-dependent killing, we need to consider the relationship between the drug concentration, the MIC, and the likelihood of selecting for drug resistance. The range of concentrations where the risk is highest is known as the mutant selection window (MSW; Blondeau *et al.*, 2001, 2004; Drlica, 2003; Hansen *et al.*, 2003; Hesje *et al.*, 2007). The MSW defines a ‘danger zone’ for therapeutic drug concentrations. This region is flanked by the MIC and the mutant prevention concentration (MPC), the latter reflecting concentrations at which the likelihood for the selection of resistant strains is minimized. However, the MSW concept is not straightforward and that what matters is not simply the duration over which drug concentrations are within the MSW but rather the proximity of these concentrations to the MPC (Ferran *et al.*, 2007).

The relationship between MIC and MPC for several pathogens and drugs were provided by Blondeau (2009). For compounds where susceptibility can be altered by mutational events, higher bioburdens increase the risk of selecting for resistant bacterial strains. Accordingly, it has recently been proposed that the MPC be based on the testing of larger bacterial inocula, that is,  $\geq 10^9$  CFU/mL, as such a high population includes bacterial subpopulations with innate resistance (spontaneous mutations, as described in particular for fluoroquinolone resistance). In so doing, the MPC can account for the probability of selection for mutant subpopulations.

At the other end of the spectrum are the events occurring when drug concentrations drop below the pathogen's MIC. Examples of undesirable subinhibitory actions of antimicrobials include as follows:

- a) Enhanced bacterial adhesion to cell surface of host (e.g., stimulation of *Staphylococcus* spp adhesion by subinhibitory concentrations of florfenicol; Blickwede *et al.*, 2004).
- b) Increase in the colony factor expression by (e.g., by moxifloxacin susceptible and resistant *Clostridium difficile*; Denève *et al.*, 2008).
- c) Rearrangement of bacterial genome (e.g., enhanced gene transfer in *Bacteroides* by tetracycline; Song *et al.*, 2009).
- d) Upregulation of virulence factors.
- e) Increase in biofilm formation, for example, as demonstrated, for example, for *Pseudomonas aeruginosa* biofilm formation that can be increased by subinhibitory concentrations of the aminoglycosides (Hoffman *et al.*, 2005). The fact that subinhibitory concentration of antimicrobials favor biofilm formation has been demonstrated for almost all veterinary-relevant bacterial species.

Conversely, subinhibitory concentrations may also have a positive clinical effect. For example, in addition to the previously mentioned case of subinhibitory concentrations of clindamycin reducing  $\beta$ -hemolysin expression in cultures of *S. aureus* (Marsik & Parisi, 1973), the biofilm and exopolysaccharide formation of *Salmonella enterica* serovar Typhimurium can be inhibited by subinhibitory concentrations of gentamicin or ciprofloxacin (Majtán *et al.*, 2008).

Additional references of that may be of interest include Rasi-gade *et al.*, 2011; Davies *et al.*, 2006; Christensen *et al.*, 2011; and Reeks *et al.*, 2005.

### Part 3: Emergence, transmission, and persistence of antimicrobial resistance

*Mechanisms of resistance and resistance transfer.* Numerous mechanisms for antimicrobial resistance have been identified, and the impact of these mechanisms appears to be both drug- and pathogen-specific (Table 2). Examples include efflux

transporters, altered protein targets, genetic mutations, and enzymatic degradation/modification of the drug that are mediated by genetic mutations or by acquired resistance genes.

When reflecting on the issue of One World–One Health and the threat of antimicrobial resistance or resistance transfer, it was concluded that antimicrobial resistance is a global ecological problem impacting the microbiome through such factors as follows:

- a) Use by the human patient: impact on commensal flora, transfer of resistant human pathogens, and the potential for transfer of resistance genes:
  - i) From interpersonal contact
  - ii) Through the environment (e.g., hospital and community)
  - iii) Through the environment (e.g., sewage and transfer by insects and rats)
  - iv) Transfer of resistant bacterial strains to and from companion animals, and *vice versa*
- b) Use on the farm: impact on commensal flora, zoonotic pathogens, and the potential for transfer of resistance genes:
  - i) Through the food chain
  - ii) Through the environment

Discussions occurring during the AAVM included such issues as the potential impact of drug use on commensal flora (Figs 1 & 2), the prevalence of the oral route of administration for collective medication, and potential mechanisms through which even systemically available drug can re-appear in the gastrointestinal tract. Complicating potential transmission between hosts is the long survival time of many pathogens on dry inanimate objects (Matlow & Morris, 2009). For example, the durations of survival for MRSA, VRE, and *C. difficile* spores are, respectively, 7 days to 7 months, 5 days to 4 months, and 5 months.

**Table 2.** Recognized resistant pathogens of human and veterinary health concern

Acronym	Definition	General information	Bacterial pathogens	Significance
ESBL	Extended spectrum $\beta$ -lactamase	Resistance to 3rd generation cephalosporins (cefotaxime resistance)	<i>Escherichia coli</i> , <i>Klebsiella</i> spp., and other Enterobacteriaceae	Resistance to most cephalosporins*
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>	Resistance to oxacillin PCR – <i>mecA</i> Chromogenic agar Cefoxitin resistance	<i>S. aureus</i>	Resistance to most $\beta$ -lactams <sup>†</sup>
VRE	Vancomycin resistant <i>Enterococcus</i>	Vancomycin screen plate PCR-vancomycin resistance genes Chromogenic agar	<i>Enterococcus</i> spp.	Resistance to vancomycin
VISA	Vancomycin intermediate resistant <i>S. aureus</i>	Reduced susceptibility to vancomycin	<i>S. aureus</i>	Reduced susceptibility to vancomycin
VRSA	Vancomycin resistant <i>S. aureus</i>	Resistance to vancomycin	<i>S. aureus</i>	Resistance to vancomycin

\*Cefotaxime, cefpodoxime, ceftriaxone, and ceftazidime.

<sup>†</sup>Penicillins, cephalosporins, carbapenems, and monobactams.

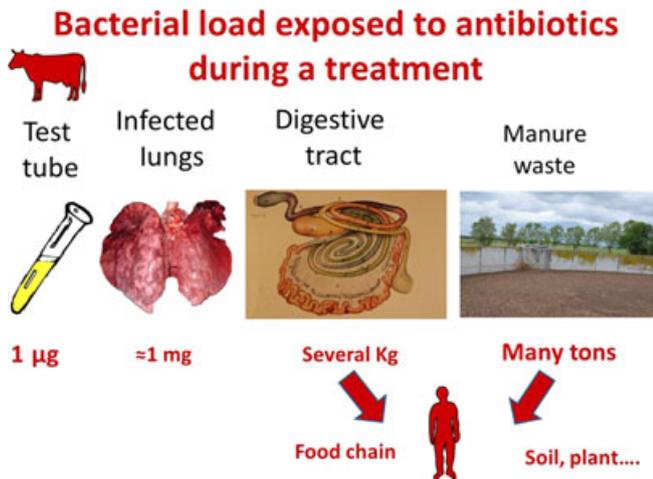


Fig. 1. Potential mechanisms for drug entry into the environment and food chain were explored through the various presentations. The recipient of the original dose can be either an animal or human patient. (From the presentation of P.-L. Toutain).

*Gut flora resistance: the impact of dosage regime.* The potential impact of metaphylaxis on gut-level resistance was studied in a rat *Klebsiella pneumoniae* lung infection model. Marbofloxacin was administered via subcutaneous injection at either 4 or 24 h after bacterial introduction into the lungs. Total daily doses were administered as a single injection or as a fractionated (4×/day) dosage regime of 16 or 64 mg/kg. Regardless of dosage regime, there was little to no impact on the susceptibility of gut *Enterococcus faecium*. In contrast, the *E. coli* population was affected by dose, with the number of isolates decreasing to a greater extent with the fractionated as compared to the single dose groups, and after 7 days of marbofloxacin treatment, a resistant *E. coli* subpopulation was found in all treatment groups. The largest percentage of rats harboring resistant intestinal *E. coli* was observed in the dose group receiving a single high-dose injection of marbofloxacin (Kesteman *et al.*, 2010).

Similarly, Looft *et al.* (2012) observed collateral effects of feeding subtherapeutic doses of antimicrobials to pigs administered medicated feed containing sulfamethazine, chlortetracycline, and penicillin in a highly controlled environment. Bacterial phylotypes shifted after 14 days of antimicrobial treatment, with the medicated pigs showing an increase in *E. coli* populations as compared to the nonmedicated pigs. Although antimicrobial resistance genes increased in abundance and diversity in the medicated swine microbiome, these investigators also noted the high background of bacterial resistance genes in the feces nonmedicated swine.

*Defining 'clinical' vs. 'biological' resistance.* The term 'resistance' can be interpreted from the perspective of epidemiological surveys (biological resistance) or from that of clinical break points. This distinction is illustrated in Fig. 3.

To appreciate the implications of any MIC value, there needs to be consistency in the methods used for its determination, and the context within the term 'resistance' is being defined. This point is illustrated by the confusion associated with 'resistance' as defined on the basis of epidemiological cutoff values (ECOFFs) vs. that defined by clinical break points. ECOFFs have been utilized to separate the naive, susceptible, wild-type bacterial population from bacterial isolates that have developed reduced susceptibility to a given antimicrobial agent (Kahlmeter *et al.*, 2003). The ECOFFs can differ from clinical break points because the latter depends not only on susceptibility data but also upon such clinically relevant data as therapeutic indication, clinical response, dosing schedules, and PK/PD relationships. Therefore, a pathogen can be clinically susceptible while not belonging to the corresponding wild population, and *vice versa*.

Differences in the parameter being measured, the *in vitro* methodologies, and how the ECOFF values are defined have confounded efforts to track global changes in microbial susceptibility patterns (Silley *et al.*, 2011). This has rendered it extremely difficult to compare resistant rates across surveillance

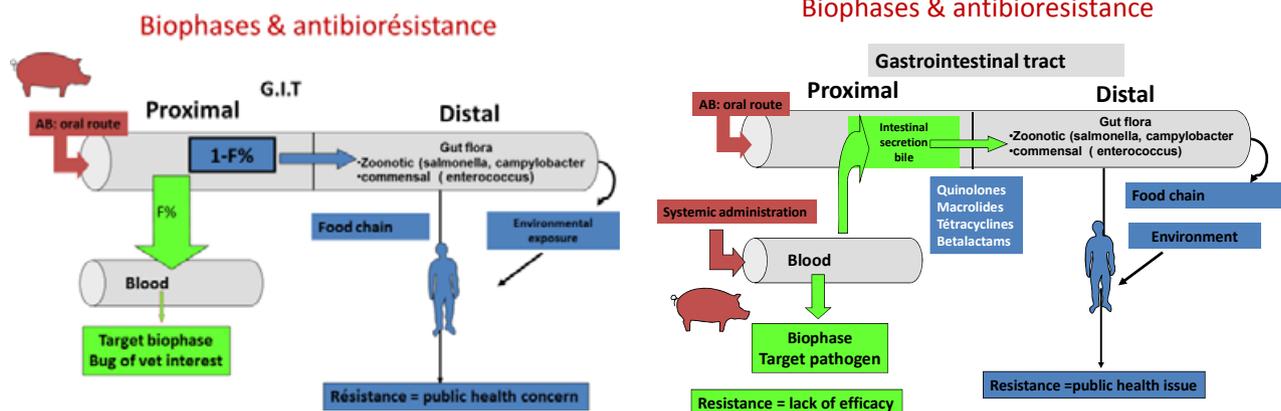


Fig. 2. Mechanisms for drug exposure in the human and animal intestine following an oral antimicrobial administration of a poorly bioavailable compound (left) or following administration of a systemically available drug, where the administered compound reenters the gastrointestinal tract through various potential excretory mechanisms (right) (From the presentation of P.-L. Toutain).

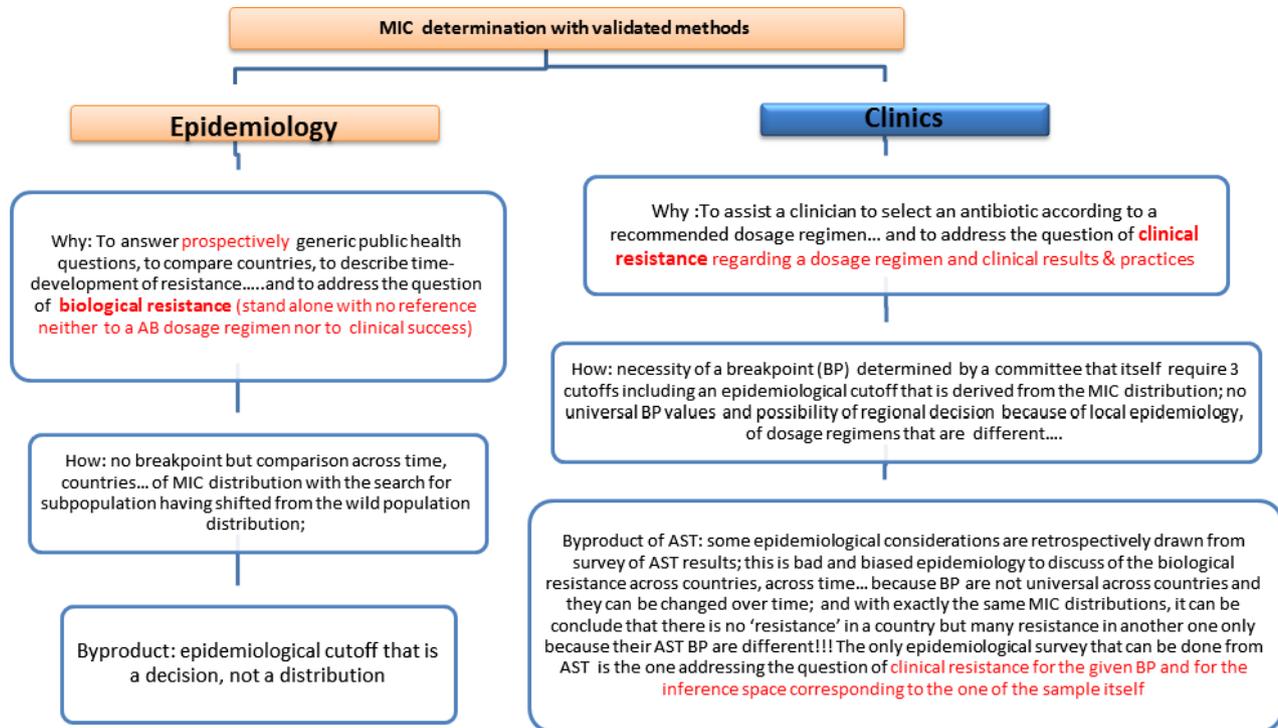


Fig. 3. Comparison of the underlying answers addressed by clinical vs. epidemiological descriptions of resistance, differences in the underlying descriptive methods, and the differences in their respective interpretations.

schemes. Moreover, there is a trend for 'resistance' in foodborne pathogens and commensals to be defined by the ECOFF values rather than by the long-established clinical break points.

Even if ECOFFs are consistently used, difference can occur with respect to data interpretation. For example, Sweden has traditionally not accepted the ECOFF used by European Food Safety Authority (EFSA) for ciprofloxacin and *E. coli* ( $>0.03 \mu\text{g/mL}$ ) because it cuts through the wild-type MIC, causing an erroneously high frequency of *E. coli* classified as resistant (Anon 8). The Netherlands and Norway have elected to set the *E. coli* ECOFF for ciprofloxacin at  $>0.06 \mu\text{g/mL}$ . Only recently has EUCAST (<http://mic.eucast.org/Eucast2/>) revised its *E. coli* ECOFF values for ciprofloxacin from  $>0.03$  to  $>0.06 \mu\text{g/mL}$ . These changes have been adopted by EFSA (Anon 1).

To insure consistency both between past and future data assessments and across antimicrobial resistance monitoring programs, it is necessary to establish a mechanism for global harmonization of methods for defining the wild-type distribution. Ultimately, owing to the numerous methodological and reporting differences that exist between monitoring and surveillance organizations, at present, it is not possible to relate resistance levels as defined by EUCAST to those defined by The European Centre for Disease Prevention and Control (EARS-Net; [http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/about\\_EARS-Net/Pages/about\\_network.aspx](http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/about_EARS-Net/Pages/about_network.aspx) or NARMS. Given the urgency of this issue, it is important to insure that epidemiological survey data are based upon comparability of sampling strategies and on methods of MIC determination.

#### Part 4: Resistance transfer between animals and humans: a focus on zoonotic pathogens

Antimicrobial Agents in Veterinary Medicine workshop discussions included a summary of the ongoing programmatic research efforts aimed at better understanding the role of human medicine vs. veterinary drug use in promoting resistance transfer in zoonotic pathogens.

*Campylobacter jejuni*. *Campylobacter* infections are responsible for 400–500 million cases of diarrhea each year worldwide. The mortality rate associated with symptomatic *Campylobacter* species infection has been estimated at 24 deaths per 10 000 culture confirmed cases, or 200 deaths per year in the USA (Ruiz-Palacios, 2007). The pathogen is commonly present in food-producing animals, especially in slaughter-age broiler chickens, and can be transmitted to humans via contaminated poultry, water, or milk.

*Campylobacter* species infection in human patients is generally associated with mild illness. These diarrheas are frequently self-curing, and in the vast majority of incidents of acute diarrhea in adults, antimicrobials are of no benefit. Most strains of *C. jejuni* and *Campylobacter coli* are susceptible to erythromycin, azithromycin, gentamicin, tetracycline, and chloramphenicol. In those cases where antimicrobials are prescribed, the treatment of choice is generally a macrolide such as erythromycin or azithromycin (Ruiz-Palacios, 2007).

Antimicrobial resistance in *Campylobacter* is mediated by multiple mechanisms depending upon the drug class. These

include antimicrobial inactivation, target mutation, efflux-mediated extrusion, and reduced uptake of the drug. When comparing across antimicrobial drug classes (tetracyclines, macrolides, and fluoroquinolones), there is a higher number of resistant *Campylobacter* isolates in poultry raised in conventional as compared to organic farms (Luangtongkum *et al.*, 2006) and increasing prevalence of fluoroquinolone resistance worldwide (Blaser & Engberg, 2008). Macrolide-resistant *Campylobacter* is less prevalent than fluoroquinolone-resistant *Campylobacter*, despite the fact that macrolides are more widely used than fluoroquinolones in animal production (Lin *et al.*, 2007). This is likely attributable to the absence of any fitness (survivability) cost associated with fluoroquinolone resistance. Therefore, fluoroquinolone resistance persists even after the selection pressure is removed (Luo *et al.*, 2005). Thus, the use of fluoroquinolones in poultry has long-term public health consequences.

**ESBL-producing *E. coli*.** Even with 'prudent use', there is the threat of selecting for ESBL-producing microbial strains through the use of  $\beta$ -lactam antibiotics (especially cephalosporins of the 3rd and 4th generations) and for an increase in the numbers of multiresistant strains through the use of fluoroquinolones (particularly in large poultry holdings). Of great concern is the apparent increase in the number of ESBL *E. coli* and *Salmonella* spp. in the absence of prior exposure to the cephalosporins, suggesting potential coselection and coresistance.

Based upon recent work, it appears that the largest transmission pathway for ESBL *E. coli* is from humans to wildlife and the environment. Guenther *et al.* (2010a,b) observed that 16% of urban rats were carriers of ESBL-producing *E. coli* and that 33% of the rats obtained from inside the sewage system located in close proximity to a university hospital carried ESBL-producing *E. coli*. Multilocus sequencing confirmed that the tested rats carried extra-intestinal pathogenic *E. coli* (ExPEC)-like strains that

typically belonged to a highly virulent lineage isolated primarily in humans and birds. This finding suggests that the urban rats may have an important role in the transmission of multiresistant and virulent *E. coli* strains (Guenther *et al.*, 2012). Resistant strains of *E. coli* were also observed in European wild birds (Guenther *et al.*, 2010b). Accordingly, wild birds might likewise constitute a potential hazard to human and animal health by transmitting multiresistant strains to waterways and other environmental sources via their fecal deposits.

There is also some transmission between humans and companion animal species and from livestock animals to humans via the consumption of raw meat, milk, and contaminated vegetables (Ewers *et al.*, 2012). In this regard, the percentages of animals carrying ESBL-positive *E. coli* strains included 4% of the dogs, 3.2% of the cats, 5.1% of the horses, and 3.3% of the cattle (Ewers *et al.*, 2010). Corresponding human data can be obtained from the European interactive database (Anon 9). Transmission pathways of ESBL and AmpC-producing *E. coli* are described in Fig. 4.

CTX-M associated ESBLs, enzymes named for their greater activity against cefotaxime than other oxyimino- $\beta$ -lactam substrates, are the most widespread type of ESBL *E. coli*. Strains relevant to human health are also being isolated with increasing frequency from companion animals, and there is global occurrence of ESBL carrying bacteria in poultry, cattle, and pigs. Nevertheless, the high similarity of major ESBL types in humans, regardless of their geographical origin, points toward person-to-person transmission as the most important route of resistance distribution rather than a transmission between animals and humans (Ewers *et al.*, 2012).

***Salmonella* spp.** *Salmonella* is an important contributor to bacterial foodborne infections, particularly in children (CDC, 2013). PCR screening of resistant isolates from food animals

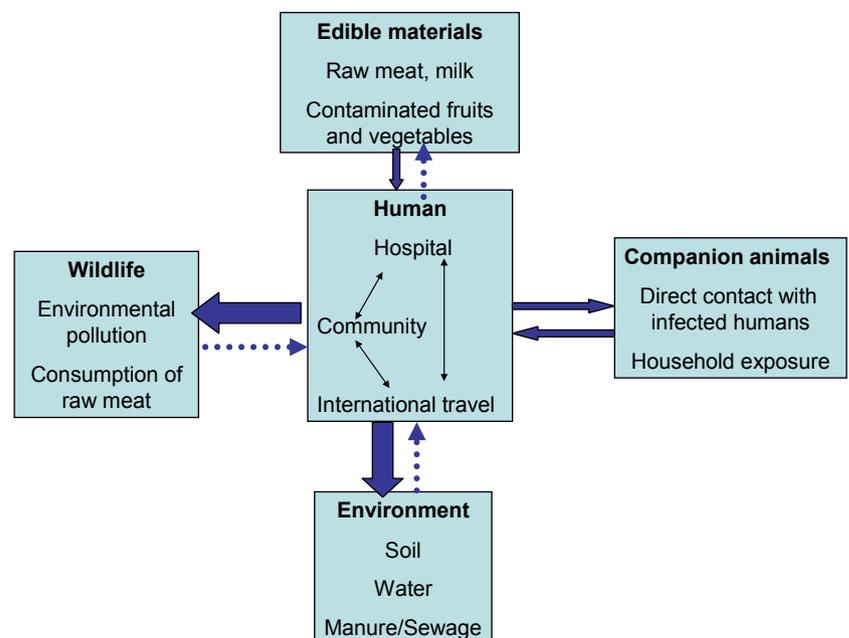


Fig. 4. Transmission pathways of extended spectrum  $\beta$ -lactamase and AmpC-producing *Escherichia coli* (based upon Ewers *et al.*, 2012).

identified these as serovars that are more often associated with invasive human infections than are most other serovars. These include typhimurium (relative proportion to all potential serotypes: 12% in humans, 3% in chickens, and 3.5% in turkeys), Heidelberg (3.5% in humans, 14.2% in chickens, and 2.4% in turkeys), and Newport (9.3% in humans, 0.8% in chickens, and 1.4% in turkeys; Fig. 5).

Antimicrobial resistance in zoonotic *Salmonella* strains is of public health concern, as genes are readily transferred from donor to recipient bacteria via plasmids (Kaldhøne *et al.*, 2008). Importantly, these plasmids frequently contain multiple resistance genes, with some of the transmissible genes appearing to facilitate *Salmonella* invasion and persistence in macrophages. Plasmids may also contain iron acquisition operators that can enhance extra-intestinal survival and increase bacterial virulence in poultry. For example, the VirB/D4 T4SS plasmids appear to increase the ability to invade and persist in macrophages. The hope is that through ongoing research, it may be possible to define factors that drive the dissemination of plasmids in enteric organisms and, in turn, identify strategies to combat their spread.

A note of interest is that while there has been much debate about the contribution of veterinary antimicrobial drug use to the overall resistance development in human pathogens, data suggest that clinical fluoroquinolone resistance in *E. coli* and nontyphoidal *Salmonella* is generally not observed (de Jong *et al.*, 2012).

#### Part 5: Premarketing approval and microbiological acceptable daily intake

Numerous scientific articles have been recently published on the importance of the relationship between gut flora and human health (e.g., Possemiers *et al.*, 2009; Human Microbiome Project Consortium, 2012; Petrof *et al.*, 2012). Because the human GI tract consists of complex microbial communities, the issue of altered human gut flora via the ingestion of antimicrobial residues in food is of potential concern.

It is well recognized that the therapeutic ingestion of antimicrobials can alter the ecology of the human intestinal flora (Jernberg *et al.*, 2010). Disruption of the normal colonization barrier is a concern when the human is exposed to the much higher concentrations associated with human therapeutic uses. This may include disruption of the colonization barrier or an

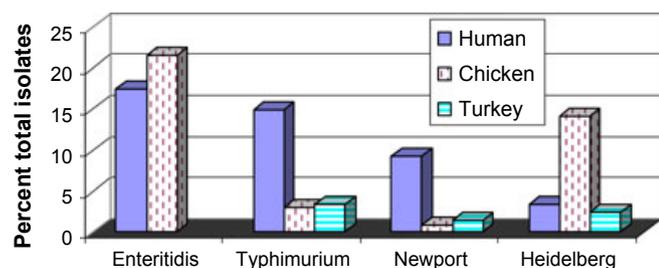


Fig. 5. Relative proportion of top serotypes in humans and poultry.

increase in the population(s) of resistant bacteria (VICH GL-36) (Anon 6).

With respect to human food safety, microbiological health concerns are raised when consumption of trace levels of antimicrobial residues in foods disrupts the human gut colonization barrier or when it leads to an increase in the population(s) of resistant bacteria. However, the question is whether the amount of drug ingested in food can reach the colon (either by being secreted in the bile, through the intestinal mucosa or reach the colon as free, unabsorbed drug) and remain biologically active. Concerns associated with effects of antimicrobial drug residues on human intestinal flora are founded on the assumptions:

- Regardless of route of administration, antimicrobials can reach the colon due to incomplete absorption or by recirculation via the bile of secretion across the intestinal mucosa.
- By virtue of their inherent antimicrobial activity, these free (active) drug concentrations are likely to inhibit at least part of the rich microbial flora of the gastrointestinal tract.

The acceptable daily intake (ADI) is a measure of the amount of a specific substance (a residue of a veterinary drug, food additive, or pesticide) in food or drinking water that can be ingested (orally) on a daily basis over a human's lifetime without incurring an appreciable health risk. When evaluating allowable antimicrobial residues in food, there are two types of ADIs to be considered: the toxicological ADI, which is based upon laboratory animal toxicology data and an estimated no observed adverse effect level (NOAEL), and the microbiological ADI (ADIm), which is based upon an assessment of drug effects on the human gut microflora. The lower of these two values is used to determine the maximum residue limit (MRL) in Europe and Canada or the tolerance in the USA. The tolerance and MRL provide the basis for establishing a withdrawal period (the time duration between the last drug intake by the animal and the allowable date for animal slaughter for human food consumption). Additional information on this evaluation procedure is provided in the VICH Guideline #36 (Anon 9). The European public MRL assessment reports are available from the EMA ([http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/vet\\_mrl\\_search.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058008d7ad](http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/vet_mrl_search.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058008d7ad)). In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has public evaluation reports of the effects of the veterinary drug residues on the human intestinal microbiota <http://www.who.int/foodsafety/chem/jecfa/publications/en/index.html>.

Although the original VICH Guideline 36 ADIm Expert Working Group readily achieved consensus on concerns pertaining to barrier disruption, the discussion at the AAVM were indicative of an ongoing scientific debate regarding the impact of antimicrobial residues on human gut flora (at the concentration range presented by ingestion of animal-derived food) as a public health concern. In part, these uncertainties are a function of the normal daily fluctuations in bacterial populations within a healthy human intestine.

- a) Jeffery *et al.* (2012) reviewed studies demonstrating that the human gut microbiota is dynamic and is subject to lifestyle-induced fluctuations. It is stated that habitual dietary intake is a major driver of microbiota composition and function. These effects of diet transcend even mammalian species boundaries; and therefore, the diet of an individual needs to be considered before attempting to categorize the gut microbiota of that individual.
- b) Wu *et al.* (2011) showed that in ten subjects aged 2–50 years old, two enterotypes were associated with diet: long-term diets enriched for protein and animal fat were associated with the *Bacteroides* enterotype, whereas diets enriched for carbohydrate were associated with the *Prevotella* enterotype. Controlled feeding over the duration of 10 days can lead to arrangement of the microbiota composition but did not lead to any change in enterotypes.

Thus, efforts to characterize the clinically relevant impact of low level drug residues are extremely complex and should be evaluated from the perspective of natural variations that occur in humans.

When determining the ADIm, the calculated MIC is based on the susceptibility of those genera for which the antimicrobial is active. Discussions focused on critical uncertainties associated with these tests. Firstly, ADIm studies will not be able to sufficiently address all of the variables that are inherent in the human gut flora, human populations, candidate molecule behavior, and other factors. Secondly, regardless of the technology or methodology employed, the results of these methods need to be placed within the context of a public health impact. Changes detectable by very sensitive and specific methods may have no consequence. In addition, the test conditions themselves are not straightforward (Ahn *et al.*, 2010; Kim *et al.*, 2011, 2012). A list of critical variables impacting the outcome from these studies is provided in Table 3 (from C. Cerniglia, National Center for Toxicological Research [NCTR], FDA).

Many attendees also emphasized that the approach to the ADIm for colonization barrier effects and resistance development are fundamentally different. For the former, it is possible to conduct simple MIC studies and/or alternative short-term *in vitro* approaches. For the latter, complex long-term *in vivo* or *in vitro* population studies are needed.

While the current guideline offers alternative approaches to addressing the ADIm, many attendees encouraged drug sponsor and regulatory authorities to dialog on appropriate study approaches, emphasizing that ‘one size does not fit all’. Requests were made regarding the need for consensus on the group of bacteria that constitute a potential public health concern [particularly when we consider the normal gut flora of each individual person may not be the same (Jeffery *et al.*, 2012)]. It was noted that the guideline itself states that ‘Preliminary information regarding the prevalence of resistance in the human intestinal flora, such as daily variation within individuals and the variation among individuals can be useful in developing criteria for evaluating resistance emergence. MIC distributions of sensitive and known resistant organisms of

**Table 3.** Overview of the methodological aspects to consider when interpreting the results *in vitro* barrier disruption studies

Methods	Aspects for consideration
Physicochemical characterization of fecal samples	Water and solid content Fecal viscosity Short-chain fatty acids pH Water-holding capacity
Drug binding/inactivation assay	Compare microbiological and analytical chemistry assays Compare fecal concentrations Compare incubation time Compare pellet vs. supernatant Compare sterilization methods Evaluate the biotransformation of the drug Predict the <i>in vitro</i> inactivation of the drug by regression analysis
Microbial community analysis of fecal samples	Characterization of the fecal microbiota by culture dependent and culture independent methods Selective plate media Flow cytometry PCR Denaturing gradient gel electrophoresis (DGGE) and pyrosequencing

concern can provide a basis to determine what drug concentration should be used in the selective agar media to enumerate resistant organisms in the fecal samples’ (VICH GL-36).

Based upon studies conducted at the NCTR and the published scientific literature, it became clear that the results and conclusions are highly dependent upon experimental methodology. Accordingly, unless we can identify relevant conditions and validate methods that will be applicable and predictive across the human population, the interpretation of studies on the effects of antimicrobial residues on the human intestinal microbiota is incompletely understood. Furthermore, the expressed opinion was that antimicrobial drug residues are inherently low due to the conservative nature of the assignment of ADIs, MRLs, and withdrawal times.

Regarding the end point of disruption of colonization barrier, the current research gaps identified by experts at the NCTR include the following:

- The need to validate and determine the predictive capabilities of *in vitro* or *in vivo* test systems to determine impact of drug residues on human intestinal microbiota and identifying potential adverse human health effects.
- The need to design research protocols that are relevant and reproducible to evaluate fecal inactivation due to drug binding in test systems to address impact of antimicrobial residues on the human intestinal microbiota.
- The need to establish databases that describe the variability of the intestinal microbiota, typically observed among or within individuals. This information is critical for determin-

- ing the magnitude of change in commensal and resistant populations (following exposure to antimicrobial drug residues in food) that may pose a risk to public health.
- d) The development of model systems that can statistically account for the inherent variability within individuals and normal intestinal microbiota.
  - e) A clearly defined end point for identifying 'disruption of colonization barrier' of the human intestinal microbiota.
  - f) A need to define the scope of representative microbiota considered sufficiently comprehensive when generating a microbial food safety assessment.
  - g) A need to identify appropriate microbial growth conditions that reflect the conditions within the human gut. Recent research from FDA–NCTR demonstrated that intestinal bacteria from normal human subjects are greatly affected by the selection of growth media. Considering that the nutritional variations in human diets function as different types of growth media, it is not surprising that intestinal microbiota fluctuate in response to diet.
  - h) The need to determine whether or not use of currently available methods for estimating the MIC is appropriate for evaluating antimicrobial drugs effect on intestinal barrier functions.

Although definitive evidence is not currently available, the data and literature (Cerniglia & Kotarski, 1999, 2005) presented at this workshop resulted in many attendees voicing the opinion that dietary consumption of microbiologically active drug residues is unlikely to either be a major factor in the development of antimicrobial resistant bacteria in the normal human intestinal flora or a cause of disruption of the normal human colonization barrier. While these issues are controversial, further consideration of the literature and research is needed for a full understanding of this complex issue.

#### *Part 6: Maintaining an affordable, safe, and abundant food supply*

By 2050, the global needs for food will increase by 70% more food. According to FAO, and as expanded upon below, 70% of these needs must come from efficiency-enhancing technologies. These include as follows:

- a) Practices – Doing it better.
- b) Products – Using new, innovative tools, and technologies.
- c) Genetics – Enhance desired traits in plants and animals.

The means and tools that should or need be used in efficiency-enhancing technologies remain, however, a matter of controversial debate. We need to 'call a truce' to the debate over the role of technology in the sustainable production of safe, affordable, and abundant food if we are to protect the three rights:

- a) Ensuring the right of all people around the world to have access to affordable food.
- b) Protecting all consumers' rights to spend their food budget on the widest variety of food choices.

- c) Creating a sustainable global food production system that does not compromise the health of humans and animals or the integrity of the environment.

The challenge of overcoming world hunger is complex and multifaceted. Allowing the entire food chain access to safe, efficiency-enhancing technologies is an essential component of a comprehensive solution to this challenge – both locally and globally. In addition, protecting the right to choose these technologies can make the dream of safe, affordable, and abundant food a reality worldwide.

As the issue of antimicrobial resistance in veterinary medicine is debated, the need to insure food availability, changes in husbandry practices necessary to meet a growing food demand, and the corresponding importance of therapeutics to insure animal health and welfare needs to be considered because animals are no longer simply considered to be 'walking food'. Ultimately, the availability of appropriate interventions for maintaining animal health and for maximizing their productivity will be a fundamental component in our ability to meet the global nutritional requirements critical for insuring public health.

#### *Part 7: Facing future challenges*

Concerns associated with the relationship between traditional antimicrobials and the propagation of resistant pathogens have led to efforts to identify alternative therapeutic targets and approaches. Much effort is currently focusing on the inhibition of virulence factors. Potential antivirulence targets have been reviewed elsewhere (Cegelski *et al.*, 2008; Escaich, 2010). Although such a therapeutic paradigm shift may have its merits, important limitations need to be considered. For example, if the drug does not influence bacterial viability, cure of infection will primarily rely upon the ability of the host immune system to clear the infection. In turn, the use of such agents as a stand-alone therapy seems to be limited to mild infections or disease prophylaxis.

Although the majority of this workshop focused on drug use in food-producing animals, issues and concerns impacting therapeutics in companion animal species were also discussed. When dealing with companion animal species, medication challenges include the absence of culture and antimicrobial susceptibility data (with poor owner compliance for follow-up cultures when they are prescribed), the lack of approved antimicrobials to cover the many unmet needs, and a paucity of information regarding the effectiveness of extra-label indications (e.g., approved drugs are usually targeted for skin and soft tissue infections). The importance of having animal species-specific drug approvals is critical for a variety of reasons including the following:

- a) The need to address the metabolic idiosyncrasies of each animal species and the potential species-specific safety concerns.
- b) Physiological differences may lead to differences both in drug PK and disease response.

- c) There is a need for body weight-appropriate animal-friendly dosage forms to encourage owner compliance with the prescribed dosage regime.

In addition to the need for drugs to treat the range of infectious diseases encountered in pets, recent publications suggest that drug resistance is an increasing challenge in companion animal medicine (JAVMA, 230, News Feb 1, 2007; van Duikeren *et al.*, 2004a,b; Jones *et al.*, 2007; Bemis *et al.*, 2009; Papich, 2012).

As we enter into a global regulatory environment, there is a need for harmonization and international collaboration. In turn, this raises questions such as:

- a) How can the regulatory bodies in different countries share data on products?
- b) Can we agree upon a single set of studies to support applications submitted to the different competent authorities (as e.g., EMA, FDA, and others)?
- c) How can we promote greater international consistency in product labeling guiding the correct and prudent use of antimicrobials?
- d) Can we agree upon criteria and test methods (including sampling protocols) for monitoring regional and global shifts in susceptibility trends over time? The need for such monitoring programs will likely increase as we strive toward improving our dosing strategies.
- e) In addition to pharmaceutical intervention, there is a need to examine potential methods for modifying husbandry practices (genetics, feeding, housing, biosecurity etc.) in an effort to reduce antimicrobial use. How we can stimulate the implementation of such advanced practices at a global level?

Finally, some attendees expressed a desire for regulatory authorities to reinstate flexible labeling-type concepts that will enable veterinarians to have the freedom to adjust doses when medically appropriate. In so doing, the practitioner can better select the dose and regimen that addresses the specific clinical situation.

## CLOSING REMARKS

Although the availability of antimicrobial therapies for animals remains essential (both for pets and for farm animals), the availability of these agents may be threatened by exaggerated perceptions of the public health impact of veterinary drug use. At least in part, this concern reflects a reliance on estimates of antibiotics tonnages used in human and veterinary medicine, even though these two estimates are not directly comparable. The veterinary community needs to be assured of the continued availability of effective treatments and the ability to prescribe the moieties necessary to treat their patients. To support this goal, the animal health community is actively involved in the development of state-of-the-art tools and approaches to assure that drugs are used in a manner consistent with public

and animal health. Efforts and needs to achieve the goals include as follows:

- a) Advanced genetic test (multilocus sequencing) methods to track the transfer of resistance elements and to understand the transmission of resistant microbial strains.
- b) PK/PD approaches to target the right dose of the right drug and for the shortest duration possible for achieving the desired therapeutic effect.
- c) Utilization of innovative drug delivery technologies and identification of novel bacterial targets.
- d) Better use of clinical laboratory susceptibility testing to insure appropriate drug selection.
- e) Antimicrobial resistance monitoring and a global harmonization of susceptibility test methods allowing consistent definitions of 'resistance'.
- f) Separation of investigations aiming to document biological vs. clinical resistance.
- g) Availability of approved drug products with appropriate dosage regimes insure the application of the right dose for the right duration.
- h) Implementation of strategies for insuring that all antimicrobial products meet the same quality control standards that are imposed during the approval of new animal drug applications.
- i) Availability of user-friendly applications (animal acceptability and convenient dosage regimes).
- j) Stimulation of basic (veterinary schools) and continuing education and continuing critical review of prescribing practices.
- k) Implementation of responsible use guidelines for antimicrobials that are practical and are translated into animal workers language.

Living in the current global environment, what happens in one part of the world will eventually impact all others. Accordingly, international guidelines for prudent use are being established. The combination of prudent/judicious use practices and surveillance/residue and microbial monitoring systems will ensure that we can meet the needs associated with animal health while avoiding a negative impact on human health care.

Without the necessary therapeutic options, we will be unable to adequately control animal diseases. As we allow our food and companion animals to serve as reservoirs for inadequately treated infections, there will be an ever-growing potential for the spread of zoonotic diseases. Considering also the demands for animal of an ever-growing human population, the animal health community is striving to insure appropriate drug in light of the common goal of 'One World Health'.

## ACKNOWLEDGMENTS

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## Declaration of interest

This workshop report is an effort to capture the rich exchange of concepts and perspectives that occurred during the 2012 AAVM. As such, it should not be construed as reflecting the opinions of any individual author or of any particular regulatory agency, organization, or company.

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