Veterinary medicine needs innovative antibiotics to fit public health requirements

Copenhagen Tran-Asap project 11-12 October 2012

P.L. Toutain
National Veterinary School;
Toulouse, France
### Medical consequences of antimicrobial resistance

**TABLE WO-1** Burden of Multidrug-Resistant (MDR) Bacteria in the European Union, Iceland, and Norway, 2007

<table>
<thead>
<tr>
<th>Human burden</th>
<th></th>
<th>Economic burden</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections (6 most frequent MDR bacteria, 4 main types of infection)</td>
<td>~400,000/year</td>
<td>Extra in-hospital costs</td>
<td>~€900 million/year</td>
</tr>
<tr>
<td>Attributable deaths</td>
<td>~25,000/year</td>
<td>Productivity losses</td>
<td>~€600 million/year</td>
</tr>
<tr>
<td>Extra hospital days</td>
<td>~2.5 million/year</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Limitation: these are underestimates.

**SOURCE:** ECDC and EMEA (2009).
The priorities of a sustainable veterinary antimicrobial therapy is related to public health issues, not to animal health issues.
The antibiotic ecosystem: one world, one health
AMR should be viewed as a global ecological problem with commensal flora as the turntable of the system.
Greening our AB

One world, one health
Tomorrow

Commensal flora
Genes of resistance
(zoonotic pathogens)

Environment

Food chain

Commensal flora

AMR should be viewed as a global ecological problem with commensal flora as the turntable of the system
A classical veterinary example of spread of AMR from animal to man and to all the ecosystem

Spread of plasmid-mediated nourseothricin resistance due to antibiotic use in animal husbandry
Dr. Ruth Hummel, H. Tschäpe, W. Witte

After using of the streptothricin antibiotic nourseothricin in animal husbandry for growth promotion, plasmid-borne resistance to streptothricin could be observed in *E. coli* from nourseothricin fed pigs, from employees in pig farms and from their family members. Moreover, streptothricin resistance plasmids also occurred in *E. coli* of man without any contact to pig farms (gut flora and even urinary tract infections). However, these individuals live in villages and towns of the territory where nourseothricin was applied to pigs.
New Eco-Evo drugs and strategies should be considered in vet medicine

The Eco-Evo concept can be defined as a perspective in which organisms are evaluated broadly in the light of evolution and ecology, rather than narrowly by the constraints of their behavior in the laboratory or in the clinical practice in relation to human or animal infections.
Is there a successful antibiotic development complying with Eco-Evo concept?
Telavancin (Telavancin is a semi-synthetic derivative of vancomycin) is a new agent (FDA approval 2009) for the treatment of Gram-positive bacteria. It is excreted primarily by renal elimination, with 60–70% of the dose excreted unchanged in the urine and <1% in the faeces. No faecal concentration of telavancin was found, which probably explains the lack of an effect on the intestinal microflora. Based on the microbiological data on the intestinal microflora as well as the results of the bioassays for antibiotic concentrations in faecal samples, telavancin has a favourable ecological profile.
Antimicrobial resistance: risk management options
Precaution principles

Veterinary antibiotics

Animal Black box

Resistance human
Prevention principle

Antibiotics

Animal
Grey box

Zoonotics
Commensals
Pathogens

AMR man
AMR man
Environment
AMR animal
The 12 concrete (EU) actions

• Actions 2 & 3: Appropriate use of AM
  – Action 2: Strengthen EU-law on veterinary medicine and medicated feed
  – Recommendation for prudent use

• Action 5: prevention of microbial infections and their spread

• **Action 7: Development of new antimicrobials or alternatives for treatment**
  – Analyse the need for new antimicrobials in vet med

• Action 8: International cooperation

• Action 10: Surveillance & monitoring

• Action 11: Research & innovation

• Action 12: Communication & education
ROUND TABLE CONFERENCE:

Action N°7 of the European Commission’s
Action Plan against the rising threats from Antimicrobial Resistance
‘Analysing the need for new antibiotics into veterinary medicine’

Hotel Metropole, Brussels
20 June 2012, 12:00 – 17:00
A number of questions for the round table

1. How to really realise a prudent use (reduced use) in practice?

2. Is there a real need for new classes of veterinary antimicrobials for animal health reasons (are essential vet antimicrobials becoming resistant?)?

2. What are the risks that the use of a new class of antimicrobials in the veterinary sector would lead to increased development of resistance of human antimicrobials?

3. If at European level would be decided that new vet antibiotics could be used, do the round table expect these would be developed by animal health industry?

4. What type of incentives could stimulate the development of new vet antimicrobials?

5. What will happen/takes place in other regions in the world on new vet antimicrobials?

Brussels, 20-06-2012
A number of questions for the round table

1. How to really realise a prudent use (reduced use) in practice
2. Is there a real need for new classes of veterinary antimicrobials for animal health reasons (are essential vet antimicrobials becoming resistant?)?
2. What are the risks that the use of a new class of antimicrobials in the veterinary sector would lead to increased development or resistance of human antimicrobials?
3. If at European level would be decided that new vet antibiotics could be used, do the round table expect these would be developed by animal health industry?
4. What type of incentives could stimulate the development of new vet antimicrobials?
5. What will happen/takes place in other regions in the world on new vet antimicrobials?
What are the drivers for discovery of novel agents in AH

1. User needs
   – Convenience, compliance, cost....

2. Unmet needs

3. Emerging disease

4. Resistance in target pathogens

5. Regulatory constraints
   – Greater scrutiny and restriction on agents in critically important categories for human uses

6. Not listed by EU:
   – Mitigate or suppress contribution of veterinary medicine to human AMR by greening veterinary antibiotics
   – New antibiotics should be developed in respect for public health ecologic concerns.
The cons against new antibiotics
The GAIN Act
(Generating Antibiotic Incentives Now)

• It focuses exclusively on bringing new antibiotics to market quickly, without any changes whatsoever to patterns of use in either human or animal populations.

• More brandy for the alcoholics (Kevin Outterson)
Should the development of new antibiotics be a public health priority

• "The development of new antibiotics without having mechanisms to insure their appropriate use is much like supplying your alcoholic patients with a finer brandy."

(Dennis Maki, IDSA meeting, 1998)
AMR is a public health priority:
But of what resistance are we speaking?
Prevent emergence of resistance:
but of what resistance?

Target pathogens

Drug efficacy in animal:
A vet issue
Possible overuse of antibiotics
Animal issue

Zoonotics

Drug efficacy in man
Natural eradication
Individual issue

Commensal flora

Resistance gene reservoir
Global ecological problem
Risk for permanent colonisation
Population issue
Q1-For AR, what are the critical veterinary ecosystems in terms of public health (commensals)
The critical animal ecosystems in terms of emergence and spreading of resistance

- Open and large ecosystems
  - Digestive tract
  - Skin
- Open but small ecosystem
  - Respiratory tract
- Closed and small ecosystem
  - Mammary gland
Bacterial load exposed to antibiotics during a treatment

- Test tube
- Infected Lungs
- Digestive tract
- Manure Sludge waste

1 µg
1 mg
Several Kg
Several tons

Food chain
Soil, plant....
Duration of exposure of bacteria exposed to antibiotics

- Test tube
- Infected Lungs
- Digestive tract
- Manure Sludge waste

24h

- Few days
- Several weeks/months

- Food chain
- Soil, plant....
Daily bacterial shedding for a grower pigs

- E coli: 7.5 g per days
- Enterococcus: about 300 µg per days

= $7.5 \times 10^6$

A 20- to 100-fold greater E. coli abundance in medicated than nonmedicated swine
Biophases & antimicrobial resistance

G.I.T

Proximal

Distal

Gut flora
- Zoonotic (salmonella, campylobacter
- Commensal (enterococcus)

AB: oral route

F%

1-F%

Blood

Target biophase
Bug of vet interest

Résistance = lack of efficacy

Résistance = public health concern

Food chain

Environmental exposure

Environmental exposure
Bioavailability of tetracyclins by oral route

• **Chlortetracycline:**
  – about 20%

• **Doxycycline:**
  – About 20%

• **Oxytetracycline:**
  – Pigs: 4.8%
  – Piglets, weaned, 10 weeks of age: by drench: 9%;
  – in medicated feed for 3 days: 3.7%.

• **Tetracycline:**
  – Pigs fasted: 23%.

• **Most of the administered dose is lost for the animal and is only spill in the environment**
Elimination of antibiotics into the environment

• As much as 75% of the antibiotics administered to food producing animals are directly excreted into the environment without any benefit for the animal
Biophases & antimicrobial resistance

Gastrointestinal tract

Proximal

Intestinal secretion

Bile

Distal

Gut flora

• Zoonotic (salmonella, campylobacter
• Commensal (enterococcus)

Systemic Administration

Quinolones

Macrolides

Tetracyclines

Blood

Food chain

Environment

Biophase

Target pathogen

Résistance = lack of efficacy

Résistance = public health issue
Consequences of antibiotic elimination by the GIT on the gut flora
Marbofloxacin impact on E. coli in pig intestinal flora
(From P. Sanders, Anses, Fougères)

- Before treatment: *E. coli* R (0.01 to 0.1%)
- After IV: Decrease of total *E. coli*, slight increase of *E. coli* R (4 to 8%)
- Back to initial level
- After repeated IM (3d): Decrease below LoD *E. coli* (2 days), fast growth (~3 \(10^6\) ufc/g 1 d). *E. coli* R followed to a slow decrease back to initial level after 12 days
Influence of a single dose of amoxicillin on the gut flora in pigs
(excretion of the bla\textsubscript{TEM} gene)
In-feed antibiotic effects on the swine intestinal microbiome

Torey Looft\textsuperscript{a,1}, Timothy A. Johnson\textsuperscript{b,c,1}, Heather K. Allen\textsuperscript{a,1}, Darrell O. Bayles\textsuperscript{a}, David P. Alt\textsuperscript{a}, Robert D. Stedtfeld\textsuperscript{b,d}, Woo Jun Sul\textsuperscript{b,c}, Tiffany M. Stedtfeld\textsuperscript{b}, Benli Chai\textsuperscript{b}, James R. Cole\textsuperscript{b}, Syed A. Hashsham\textsuperscript{b,d}, James M. Tiedje\textsuperscript{b,c,2}, and Thad B. Stanton\textsuperscript{a,2}

\textsuperscript{a}Agricultural Research Service, National Animal Disease Center, US Department of Agriculture, Ames, IA 50010; and \textsuperscript{b}Center for Microbial Ecology, \textsuperscript{c}Department of Crop and Soil Science, and \textsuperscript{d}Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI 48823

- Performance-enhancing antibiotics (old antibiotics)
  - chlortetracycline, sulfamethazine, and penicillin

- Phylogenetic, metagenomic, and quantitative PCR-based approaches to address the impact of antibiotics on the swine gut microbiota
• It was shown that antibiotic resistance genes increased in abundance and diversity in the medicated swine **microbiome** despite a high background of resistance genes in nonmedicated swine.

• Some enriched genes, demonstrated the potential for indirect selection of resistance to classes of antibiotics not fed.
Horizontal exchanges of genes of resistance between commensal bacteria and pathogens

Gram négatif

Pseudomonas

Enterobacteriaceae

Vibrio cholerae

Campylobacter

Gram positif

Staphyloccoci

Enterococci

Pneumococci

Streptococci
“Classical” natural history of bacterial infections

Andremont et al, The lancet infection 2011 11 6-8
« New » natural history of bacterial infections

Commensal flora of a future patient

Colonization/carriage
Gene of resistance
ESBL, CTX-M…

Dissemination of genes of resistance

Individual low probability
Delayed (Months, years)

Specific pathogen

Disease

Ecological amplification of MDR

the commensal genetic pool is large and encompasses the potential for many different mechanisms conferring AMR

Adapted from Andremont et al, The lancet infection 2011 11 6-8
Impact of primary care antibiotic prescription on the risk of acquisition of AMPR organisms in individual patients

• A pooled odds ratio for antimicrobial resistance in a patient presenting with a **urinary tract infection** as 2.5 (95% confidence interval [CI] 2.1–2.9) within two months of antibiotic treatment, falling to 1.3 (95% CI 1.2–1.5) within 12 months.

• For **respiratory tract** bacteria, the equivalent pooled odds ratios were 2.4 (95% CI 1.4–3.9) and 2.4 (95% CI 1.3–4.5) for the same periods, respectively

Stewardson et al: Current Opinion in Pharmacology 2011, 11:446–452
Q3: What are the transmission pathways between animals and man
Pathways of transmission of AMR between animals and man

- Slaughter house
- Meat
- Environment
  - Soil
  - Water
  - Air

3 possible pathways
Feces are the main vehicle for transmission of AMR from animal to man

Transportation of poultry

- *Campylobacter*: Top to bottom contamination by feces during transportation

![Figure 1. Chickens held in crates before being processed. Note that the bottom crate is empty.](image1)

*Campylobacter: prevalence*

The food chain is a critical pathway for resistance transmission of resistance from animal to man

Prevalence: 60-100% in feces

Prevalence: 0-5% for meat

Prevalence: 0-32% for carcass

![Figure 7. A commercially available rotary scald tank. The carcasses are placed on a blade which is then rotated several times through the heated water.](image2)

![Figure 10. Examples of complete (A) and incomplete (B) plucking of chicken carcasses.](image3)

Air, water & ground pollution
Desirable properties of a veterinary antibiotic from an Eco-Evo perspective
R&D: PK selectivity of antibiotics for clean feces

Proximal

1-F=90%

Oral

F=100%

Blood

Kidney

Biophase

Animal health

Distal

Gut flora
• Zoonotic (salmonella, campylobacter)
• Commensal (enterococcus)

Food chain
Quinolones, macrolides

Environment

Résistance = public health concern
Hazard associated to the release of antibiotic in the environment

Digestive tract

Manure Sludge waste

Several Kg

Several tons

Food chain

Soil, plant....
Fate of antibiotics, zoonotic pathogens and resistance genes: residence time in the different biotopes

Digestive tract: 48h

Lagoon: few weeks

Ex: T1/2 tiamuline = 180 days

Air, water & ground pollution

Bio-aérosol

Air pollution
Feces production

• in 2002, 185 million head of swine were sold in the United States, generating approximately $280 \times 10^6$ tonnes of fresh manure annually.

• Chicken production in the United States in 2006 was estimated at nearly 9 billion head, generating approximately $460 \times 10^6$ tonnes of manure.

• Beef cattle estimates in the United States in 2007 were 33.3 million head producing approximately $360 \times 10^6$ tonnes of manure

• **About 3.5 tons of feces per american per year**
Persistence of Antibiotics in Manure
## Rate of antibiotic degradation in manure, soil, waste...

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Matrix</th>
<th>Dégradation %</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortétracycline</td>
<td>Cattle manure</td>
<td>24</td>
<td>84</td>
</tr>
<tr>
<td>Tétracycline</td>
<td>Pig manure</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>Soil+contam manure</td>
<td>0</td>
<td>180</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>Sediment slurry, aéobiose</td>
<td>50</td>
<td>43</td>
</tr>
<tr>
<td>TMP</td>
<td>Sewage sludge</td>
<td>50</td>
<td>22-41</td>
</tr>
<tr>
<td>Sulfamides</td>
<td>Manure/sludge</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>manure</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Tiamulin</td>
<td></td>
<td>50</td>
<td>26</td>
</tr>
<tr>
<td>Tylosine</td>
<td>Pig manure, anaerobic</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Sandy loam &amp; manure</td>
<td>77</td>
<td>30</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>Cattle manure</td>
<td>&lt;1</td>
<td>56</td>
</tr>
</tbody>
</table>
Accumulation in the environment of mobile genetic elements
Accumulation in the environment of mobile genetic elements

• Not only resistant bacteria should be considered units of epidemiological surveillance of AMR, all other elements (pieces) able to shape the natural evolutionary history of AMR should also be considered.

• Consequently, all these biological-genetic elements, at any level of the hierarchy, should become targets for intervention against AbR.
Evidence of Increasing Antibiotic Resistance Gene Abundances in Archived Soils since 1940

CHARLES W. KNAPP,†* JAN DOLFING,†
PHILLIP A. I. EHLE RT, † AND
DAVID W. GRAHAM*†

Persistence of Bacteria during Manure Storage
Decline of inoculated *S. enterica* (●), *E. coli* O157 (○), *L. monocytogenes* (▼), and *C. jejuni* (△) introduced into fresh dairy cattle slurry and spread onto a sandy loam soil in late spring

Selective Pressure of Antibiotic Pollution on Bacteria of Importance to Public Health
• Measured environmental concentrations in river sediments, swine feces lagoons, liquid manure, and farmed soil inhibit wild-type populations in up to 60%, 92%, 100%, and 30% of bacterial genera, respectively.

• At concentrations used as action limits in environmental risk assessment, erythromycin and ciprofloxacin were estimated to inhibit wild-type populations in up to 25% and 76% of bacterial genera.
Selective Pressure of Antibiotic Pollution on Bacteria of Importance to Public Health

• Measured environmental concentrations of antibiotics, as well as concentrations representing environmental risk assessment action limits, are high enough to exert a selective pressure on clinically relevant bacteria that may lead to an increase in the prevalence of resistance.

Tello et al; EHP 120:1100–1106 (2012).
The Eco-Evo approach: cure the environment

- By extension, other environments that can be successfully treated are farms, fish factories, and eventually water effluents. Indeed, the notion of an “ill environment” should be increasingly encouraged, and medical treatment-like approaches might be increasingly applied to prevent and cure biologically altered environments.
II: Risk management options
1-Development of non-antibiotic substances for prophylaxis
Terms to describe herd or flock antibiotic use

Disease

Therapy

Metaphylaxis (Control)

Prophylaxis (prévention)

Growth promotion

Administration of an AB to an animal, or group of animals, which exhibit clinical disease

Administration of an AB to animals, usually as a herd or flock, in which morbidity and/or mortality has exceeded baseline norms. Hazard present

• Administration of an AB to exposed healthy animals considered to be at risk, (before onset of disease)
• Risk factor present

Administration of an antimicrobial, usually as a feed additive, to growing animals that results in improved physiological performance.
Drug repurposing from an academic perspective

• The rationale behind is that de novo drug discovery is a lengthy and costly process, whereas already approved drugs are more probably to be repurposed for another indication.

• No bad surprise in terms of safety, withdrawal time.....
The case of tolfenamic acid

- Tolfenamic acid is an NSAID
- Tolfenamic acid as a competitive inhibitor (Ki 26 µM) of the binding of pathogenic hantavirus to Decay Accelerating Factor (DAF/CD55)
- Its typical serum concentration, around 20 micromoles/L in plasma
The tolfenamic acid as a competitor of the DAF: conclusion

- Its properties make this drug a suitable target for treating infections by a wide range of pathogens.
Therapeutic alternatives to antibiotics

1. Vaccines
2. Bacteriophages
3. Bacteriocins
4. Disruption of the « quorum sensing » & of virulent factors
5. Interférence avec les facteurs d’attachement
6. Antibody
7. Probiotics & Prebiotics
8. Anti-inflammatory drugs
9. Phytotherapy & othe natural substances (honney...)
10. Chemical Additives (ZnO2....)
11. Homeopathy
We need new antibiotic eco-friendly with less implications for human health

• These new antibiotics should be developed in respect for public health ecologic concerns.

• They should not influence the gut flora so to avoid the contamination of the environment with resistant bacteria.

• This implies to develop antibiotics having a good selectivity for parenteral and oral use.
Selectivity of antimicrobial drugs in veterinary medicine

Selectivity

PD

Narrow spectrum

PK

Selective distribution of the AB to its biophase
Pharmacodynamic selectivity

• **Narrow spectrum**
  - Gram positive vs. Gram negative
  - Advantages
    • Limit the risk of AMR
  - Limites
    • Segmentation of the market
    • Require an accurate diagnostic
Selectivity of antimicrobial drugs in veterinary medicine

Selectivity

PD

Narrow spectrum

PK

Selective distribution of the AB to its biophase
Innovation: PK selectivity of antibiotics

- **Proximal**
  - **Blood**
  - **Kidney**
  - **Efflux**

- **Distal**
  - **G.I.T.**
  - **Gut flora**
    - Zoonotic (salmonella, campylobacter)
    - Commensal (enterococcus)

- **Biophase**
  - **IM**

- **Food chain**
  - Quinolones, macrolides

- **Environment**

- **Resistance = public health concern**

- **Animal health**
My view of an ideal antibiotic for vet medicine

<table>
<thead>
<tr>
<th>High plasma clearance</th>
<th>Rapidly metabolized (in vivo, environment) to inactive metabolite(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High renal clearance</td>
<td>Elimination by non-GIT route (not bile or enterocyte efflux)</td>
</tr>
<tr>
<td>Low biliary clearance</td>
<td>Pathogens are extracellular; half-life rather short; not too short to compensate a relatively high clearance</td>
</tr>
<tr>
<td>No efflux by PGP</td>
<td></td>
</tr>
<tr>
<td>volume of distribution not too high</td>
<td></td>
</tr>
<tr>
<td>High bioavailability by oral route</td>
<td>To avoid to expose distal GIT to active AB</td>
</tr>
<tr>
<td>Low binding to plasma protein</td>
<td>Only free antibiotic is active; to reduce the possible nominal dosage regimen and environmental load</td>
</tr>
<tr>
<td>High binding to cellulosis</td>
<td>To inactivate AB in large GIT</td>
</tr>
<tr>
<td>Appropriate degradability</td>
<td>Rapid inactivation in the environment</td>
</tr>
<tr>
<td>High potency</td>
<td>To be able to select a low dose</td>
</tr>
<tr>
<td>High PK selectivity (biophase)</td>
<td>To distribute only to target biophase</td>
</tr>
</tbody>
</table>
A long half-life (HL) is desirable for convenience in vet medicine: two possible options

**Long HL**

**A substance property**
- Low clearance
  - High MW
  - Lipophilic
    - Likely lower degradability
    - Excretion by the GIT
- Large volume of distribution
- Large diffusion

**A formulation property**
- Slow absorption (flip-flop)
  - High clearance
  - Hydrophilic
    - Likely higher degradability
    - Excretion by the kidney

**Macrolides/FQ**

**Beta-lactams/sulfonamides**
Objective 1: Improve the oral bioavailability for oral antibiotics
Prodrugs: Modification of the extent of bioavailability of ampicillin

- **Ampicillin**
  - Log P of 0.57 and F=30-50%
  - Incorporation of hydrophobic substituents or by masking polar groups such as alcohol or acids (with esterification to alkylation)

- **Bacampicillin** (log P=2.04) and **pivampicillin** (log P=2.44)
  - are prodrug having the polar carboxylic of ampicillin masked by a metabolically labile ester;
  - the net outcome is that this ester are satisfactory absorbed frm the gut; the labile ester linkage is then cleaved during the fist-pass metabolism in the liver
Prediction of an appropriate oral bioavailability
The ‘rule of 5’ and its implementation

• The ‘rule of 5’ states that: **poor absorption or permeation are more likely when:**
  – There are more than 5 H-bond donors (expressed as the sum of OHs and NHs);
  – The MWT is over 500;
  – The Log P is over 5 (or MLogP is over 4.15);
  – There are more than 10 H-bond acceptors (expressed as the sum of Ns and Os)
  – Polar surface area greater than 140 Å²
Objective 2: Degradation or inactivation of AB in the digestive tract
The use of recombinant β-lactamase to degrade a β-lactam antibiotic in the jejunum
Reduction of selective environments for high-risk resistant clones

• The strategy of destroying antibiotics in the intestine by the uptake of specific detoxifying enzymes, as betalactamases in patients treated with beta-lactam agents, or antibiotic-binding substances has been proposed (http://www.davolterra.com/rd-pipeline).
Enzymic degradation of a β-lactam antibiotic, ampicillin, in the gut: a novel treatment modality

Jaana Harmoinen¹*, Kirsi Vaali², Pertti Koski³, Kaisa Syrjänen³, Outi Laitinen¹, Kai Lindevall³ and Elias Westermark¹
Degradation of ampicillin in the gut by β-lactamase: a dose effect relationship

**Figure 1.** Effect of orally administered β-lactamase pellets on the serum ampicillin level in beagle dogs. Different doses of encapsulated enteric-coated β-lactamase pellets were given orally 3 min before iv-administered ampicillin (40 mg/kg). The values for each test group [white stars, ampicillin 40 mg/kg iv + placebo per os (n = 6); white squares, ampicillin 40 mg/kg iv + TRBL 0.003 mg/kg per os (n = 5); black squares, ampicillin 40 mg/kg iv + TRBL 0.03 mg/kg per os (n = 6); black stars, ampicillin 40 mg/kg iv + TRBL 0.3 mg/kg per os (n = 5)] are presented as mean serum ampicillin concentrations ± S.E.M. at different time points.

**Figure 2.** Effect of orally administered β-lactamase pellets on the concentrations of ampicillin in the jejunum of beagle dogs after iv administration of ampicillin (40 mg/kg). The values for each test group [white stars, ampicillin 40 mg/kg iv + placebo per os (n = 6); white squares, ampicillin 40 mg/kg iv + TRBL 0.003 mg/kg per os (n = 5); black squares, ampicillin 40 mg/kg iv + TRBL 0.03 mg/kg per os (n = 6); black stars, ampicillin 40 mg/kg iv + TRBL 0.3 mg/kg per os (n = 5)] are presented as mean jejunal ampicillin concentrations ± S.E.M. at different time points.
Effects of enterocoated Beta-lactamase pellets on the % of strains of coliform isolates from different treatment group resistant to ampicillin

FIG. 4. Effects of enterocoated β-lactamase pellets on the proportion of strains of coliform isolates from different treatment groups resistant to ampicillin (10 µg) during the experimental period. The duration of the treatment period was 14 days. Light gray bars, ampicillin plus TRBL; dark gray bars, ampicillin plus placebo; open bars, placebo i.v. and placebo p.o.
Objective 3: Degradation or inactivation of AB in the environment
Residual microbiological activity of ceftiofur (%) in the urine and in a urine+feces mixture of bovine treated with ceftiofur at different interval of incubation

<table>
<thead>
<tr>
<th>Time</th>
<th>Urine</th>
<th>Urine + feces (1:1 mixture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>24</td>
<td>63.4</td>
<td>42.6</td>
</tr>
<tr>
<td>48</td>
<td>42.2</td>
<td>ND</td>
</tr>
<tr>
<td>72</td>
<td>23.1</td>
<td>16.5</td>
</tr>
<tr>
<td>144</td>
<td>2.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Half-life</td>
<td>23h</td>
<td>17h</td>
</tr>
</tbody>
</table>

Observation of intestinal metabolism of ceftiofur to antimicrobially inactive metabolites suggests that urinary excretion of the drug is the primary concern for environmental contamination.

Adapted from J. Agric. Food Chem. 1990, 38, 890-894
Ceftiofur: degradation in Feces.

J. Agric. Food Chem. 1990, 38, 890-894

- Fecal materials contain microorganisms capable of degrading ceftiofur to non microbiologically active materials
- The degradation of ceftiofur in sterile feces is slower compared to normal feces, suggesting the role microorganisms play in this degradation
Degradation of ceftiofur in soils

- The half-life values at 50% for glucose in Florida, California, and Wisconsin soil were 2.0, 2.8, and 7.6 days respectively, whereas for ceftiofur sodium these were >49.0, 22.2, and 41.4 days, respectively

Figure 5. Aerobic degradation of glucose and ceftiofur in soils.
Objective 4:
Selection of renally eliminated substances
The % of urinary excretion decreased or fecal excretion increased with increasing octanol±water partition coefficient, especially for the drugs with C log P>0.

- The more hydrophobic is a drug, the more likely it is to be excreted in the feces.

*Figure 1.* Dependence of urinary excretion of drug-related material following intravenous administration on C log P.
**Predominant routes of drug elimination by BCS class**

<table>
<thead>
<tr>
<th>High Solubility</th>
<th>Low Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class 1</strong></td>
<td><strong>Class 2</strong></td>
</tr>
<tr>
<td>Metabolism</td>
<td>Metabolism</td>
</tr>
<tr>
<td><strong>Class 3</strong></td>
<td><strong>Class 4</strong></td>
</tr>
<tr>
<td>Renal and/or Biliary Elimination of Unchanged Drug</td>
<td>Renal and/or Biliary Elimination of Unchanged Drug</td>
</tr>
</tbody>
</table>

Fig. 2. Predominant routes of drug elimination for drug substances by BCS class.

- Class 1 and Class 2 compounds are eliminated primarily via metabolism, whereas Class 3 and Class 4 compounds are primarily eliminated unchanged into the urine and bile.
## Renal clearance of different quinolones

<table>
<thead>
<tr>
<th>Drugs</th>
<th>% of total clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>80-95%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>65</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>50</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>13</td>
</tr>
<tr>
<td>Grepafloxacin</td>
<td>10</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>5-10</td>
</tr>
</tbody>
</table>

Adapted from Hooper DC CID 2000;30:243-254
Objective 4: 
Selection of substances having a low intestinal clearance
Major ABC transporters (P-GP, BCRP and MRP2) involved in the interaction with FQ

- Gastrointestinal secretion of fluoroquinolones represents a quantitatively important route for the clearance of these drugs.
Danofloxacin in pigs: efflux in the gut after an IV administration

TABLE 3: Selected pharmacokinetic variables for danofloxacin in various parts of the intestine and in lymph nodes after intravenous administration of 2-40 mg kg⁻¹ bwt. to healthy pigs.

<table>
<thead>
<tr>
<th></th>
<th>AUC (µg h g⁻¹)</th>
<th>AUC/AUCplasma</th>
<th>MRT (h)</th>
<th>Cmax (µg ml⁻¹)</th>
<th>Tmax (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-total</td>
<td>43.23</td>
<td>10.73</td>
<td>12.93</td>
<td>3.52</td>
<td>2.0</td>
</tr>
<tr>
<td>-mucosa</td>
<td>47.14</td>
<td>11.70</td>
<td>12.49</td>
<td>3.89</td>
<td>2.0</td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-total</td>
<td>44.20</td>
<td>10.97</td>
<td>9.86</td>
<td>4.46</td>
<td>2.0</td>
</tr>
<tr>
<td>-mucosa</td>
<td>61.76</td>
<td>15.33</td>
<td>10.37</td>
<td>5.08</td>
<td>2.0</td>
</tr>
<tr>
<td>-content</td>
<td>400.65</td>
<td>99.42</td>
<td>8.58</td>
<td>28.81</td>
<td>2.0</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-total</td>
<td>39.44</td>
<td>9.79</td>
<td>10.01</td>
<td>2.96</td>
<td>2.0</td>
</tr>
<tr>
<td>-mucosa</td>
<td>51.35</td>
<td>12.74</td>
<td>10.75</td>
<td>4.07</td>
<td>2.0</td>
</tr>
<tr>
<td>-content</td>
<td>213.93</td>
<td>53.08</td>
<td>8.79</td>
<td>17.19</td>
<td>2.0</td>
</tr>
<tr>
<td>Caecum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-total</td>
<td>75.88</td>
<td>18.83</td>
<td>29.85</td>
<td>2.43</td>
<td>2.0</td>
</tr>
<tr>
<td>-mucosa</td>
<td>115.83</td>
<td>28.74</td>
<td>26.04</td>
<td>4.44</td>
<td>6.0</td>
</tr>
<tr>
<td>-content</td>
<td>375.06</td>
<td>93.07</td>
<td>38.21</td>
<td>9.97</td>
<td>6.0</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-total</td>
<td>93.59</td>
<td>23.22</td>
<td>50.40</td>
<td>2.40</td>
<td>2.0</td>
</tr>
<tr>
<td>-mucosa</td>
<td>82.18a</td>
<td>20.39</td>
<td>-</td>
<td>5.24</td>
<td>24.0</td>
</tr>
<tr>
<td>-content</td>
<td>211.02a</td>
<td>52.36</td>
<td>-</td>
<td>20.94</td>
<td>24.0</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>42.91</td>
<td>11.28</td>
<td>10.65</td>
<td>3.82</td>
<td>2.0</td>
</tr>
</tbody>
</table>

a: AUC-values for colon mucosa and content are calculated without λ.
AUC = area under curve; MRT = mean residence time; Cmax = peak concentration; Tmax = time at peak concentration.
Enterocytes transporters

- ABC transporters, including MDR1 (P-gp), MRP2, and BCRP, are found on the apical membrane, where they either limit the intestinal uptake of their substrates or contribute to the active secretion of drugs from the blood to the intestinal lumen.
Economical aspects
Bottleneck for discovery of new AB in veterinary medicine

1980-1990
Human Health target amenable to Animal Health use

HH: 2000
Remaining HH projects targeting resistant pathogen with unique market

AH: Present
Eco-evo drugs are needed
AH will be on their own
Regulatory considerations
Incentives For New Drug Development: patent extension

• Patent Term Extension for important antibiotics with “high therapeutic potential
  – Extension of the period of effective patent life granted to new antibiotics. Drug patents have a statutory lifetime of twenty years, but the effective patent length is shorter because of the amount of time the drug approval process takes
Impact of the CVMP guidelines

The impact of the CVMP antibiotic guidelines on research and development of antibiotics

Alexander Böttner*

Intervet Innovation GmbH, Schwabenheim, Germany
Clinical trials: non-inferiority

- No longer acceptable
  - Promotion of me-too drugs
  - For human medicine FDA require superiority trials for antibiotics used to treat self-resolving nonlethal infections

- We need “superiority trials” with relevant endpoints
  - Ecological impact of the new antibiotic
VICH phase I not conservative enough

• if the predicted environmental concentration in soil is < 100 ppb (i.e., soil action limit) experimental results suggest that the VICH phase I soil action limit for veterinary medicines does not protect background antibiotic resistance levels.

• Certain antibiotics at concentrations < 100 ppb may inhibit a significant fraction of clinically relevant bacteria in the environment
VICH phase I not conservative enough

- Experimental results suggest that VICH phase I action limits leave an ample margin for antibiotics to exert a selective pressure on bacteria of clinical importance in the environment