European College of Veterinary Pharmacology and Toxicology
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Antimicrobial Therapy in Animals and Public Health.

Early Treatments With Inoculum-Size-Adjusted Doses to Conciliate Control of Infectious Diseases, Reduction of Antibiotic Consumption and Prevention of Antimicrobial Resistance in Commensal Flora

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Medical consequences of antimicrobial resistance in Humans

**Europe**
More than 25,000 deaths per year
More than 1.5 billion Euros

**US**
About 23,000 deaths per year
One World, one Health: bacterial ecosystems
Public health concerns are becoming the priorities of a sustainable veterinary antimicrobial therapy.
Public health concerns for antibiotics in animals

Gram-positive bacteria

Meticillin resistant *Staphylococcus aureus* (MRSA)
- Livestock-associated (LA-MRSA), horse, pets
- Direct contact: professionals, owners
- Clonal spread, transient carriages in humans
- To date, limited ability to spread into human population (in the future?)

Role of the pharyngeal flora
Public health concerns for antibiotics in animals

Gram-negative bacteria

Zoonotic foodborne pathogens
- *Salmonella, Campylobacter*
- Food safety
- Self-limiting infections: no inter-human transmission
- Infections generally managed without antimicrobial therapy
Extended-spectrum $\beta$-lactamase (ESBL) carrying Enterobacteria

- ALL enterobacteria: « vehicle » of antimicrobial resistance genes
- Clonal + HORIZONTAL transmission (plasmids)
- Spread to the resident flora: inter-bacteria exchanges
- Spread to the human population: inter-human exchanges
Extended-spectrum \(\beta\)-lactamase (ESBL) carrying Enterobacteria

- ALL enterobacteria: « vehicle » of antimicrobial resistance genes
- Clonal + HORIZONTAL transmission (plasmids)
- Spread to the resident flora: inter-bacteria exchanges
- Spread to the human population: inter-human exchanges

The gut is the epicentre of antibiotic resistance

Jean Carlet
Critical bacterial flora for antimicrobial resistance

Digestive tract

Proximal

Distal

Zoonotic pathogens (*Salmonella*, *Campylobacter* …)
Commensal flora (resistance genes)

Blood

Infectious site
Pathogens of veterinary interest

Animal Health

HUMAN

Human Health

AB: parenteral route

AB: oral route

Contact

Food chain

Environment

HUMAN

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Critical bacterial flora for antimicrobial resistance

Digestive tract

Proximal

Distal

Zoonotic pathogens (Salmonella, Campylobacter ...)

Commensal flora (resistance genes)

Blood

AB: parenteral route

AB: oral route

Infectious site
Pathogens of veterinary interest

ANIMAL HEALTH

HUMAN HEALTH

Food chain
Environment

Contact

HUMAN

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The current recommendations in human medicine
Current recommendation in human medicine

- Higher [efficacy / resistance prevention] are obtained with **HIGHER antibiotic daily doses**:
  - Fluoroquinolones: Ciprofloxacin, Levofloxacin …
  - Beta-lactams: Amoxicillin, Cephalosporins, Penems
  - Macrolides

- High density bacterial loads harbour sub-populations of reduced susceptibility:
  - **Resistance** / Spontaneous mutations
  - **Tolerance** / Persisters
Current recommendation in human medicine

**Fluoroquinolones:** Resistances occur by random mutation \((10^{-9})\) on genes coding for FQ targets (DNA gyrase).

**Many classes of AB:** Subpopulations with lower susceptibility (resistance, tolerance) appear in high density bacterial loads.
Fluoroquinolones: Resistances occur by random mutation \((10^{-9})\) on genes coding for FQ targets (DNA gyrase)

Current recommendation in human medicine

« Hit hard and fast ... »

« Hit hard »

with lower susceptibility (resistance, high density bacterial loads)
Current recommendation in human medicine

Treatment durations are too long (acute infections)

- **Equivalent clinical success** for pneumonia
  - 500 mg levofloxacin 10 days
  - 750 mg levofloxacin 5 days
  Dunbar et al. CID 2003:37 752-760

- **Equivalent clinical success** for acute exacerbations of chronic bronchitis
  - META-ANALYSIS: FLUOROQUINOLONES, BETA-LACTAMS, MACROLIDES
  - 5 days versus 7-10 days
  Falagas et al. JAC 2008:62 442-450
    - AMOXICILLIN – CLAVULANIC ACID
    - 3 days versus 10 days

- **Lower resistance selection** in commensal pharyngeal flora
  - PENICILLIN, AMOXICILLIN, MACROLIDES
  - With **lower durations** of treatments
  Schrag et al. JAMA 2001:286 49-56
  Guillemot et al. JAMA 1998:279 365-370
  Kastner & Guggenbichler Infection 2001:5 251-256
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    - AMOXICILLIN – CLAVULANIC ACID
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- **Lower resistance selection** in commensal pharyngeal flora

« Hit hard and fast ... then leave as soon as possible »
  - with lower durations of treatments
  Schrag et al. JAMA 2001:286 49-56
  Guillemot et al. JAMA 1998:279 365-370
  Kastner & Guggenbichler Infection 2001:5 251-256

« Hit hard and stop early »

File TM, Clinical cornerstone 2003 S3 (S21-S28)
Digestive tract

Proximal

Blood

Distal

Zoonotic pathogens (Salmonella, Campylobacter …)
Commensal flora (resistance genes )

Infectious site
Pathogens of veterinary interest

ANIMAL HEALTH

AB : parenteral route
AB : oral route

« Hit hard … »

Unfavourable ?

Favourable

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ANIMAL HEALTH

Infectious site
Pathogens of veterinary interest

AB : parenteral route

AB : oral route

Digestive tract

Proximal

Blood

Distal

Zoonotic pathogens (Salmonella, Campylobacter ...)
Commensal flora (resistance genes)

Favourable?

« ... leave asap »

« Hit hard ... »

Favourable
A strategy tailored to antimicrobial therapy in food animals?
Antibiotics in food animals

Hypothesis: The size of the bacterial load at the infectious site influences antimicrobial efficacy.

- Curative treatment of sick animals
- Metaphylaxis (control) Treatment of all the group
- Prophylaxis Prevention

The same dose?

Disease
- High bacterial load (infectious site)
- Symptoms
- No or no growth

Health
- No symptoms
Inoculum size influences antimicrobial activity

1. Clinical and microbiological cure?
   1. *In vitro* evidences of the effect of inoculum size on antimicrobial activity
   2. *In vivo* evidences

2. Resistance selection/prevention at the infection site?
Inoculum size and *in vitro* susceptibility assessment

MICs estimated with different inoculum densities, relative to the MICs at $2 \times 10^5$

- Ciprofloxacin
- Gentamicin
- Linezolid
- Daptomycin
- Oxacillin
- Vancomycin

*Figure 3.* MICs estimated with different inoculum densities, relative to that MIC at $2 \times 10^5$. These estimates were obtained from cfu data; when the viable cell density at 18 h was approximately equal to that in the initial inoculum.
Inoculum size and *in vitro* antimicrobial activity

- Ciprofloxacin and imipenem against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Mizunaga et al. JAC 2005)

  ![Ciprofloxacin](image1)

  ![Imipenem](image2)

  - 10^8 CFU/mL
  - 10^6 CFU/mL

- Marbofloxacin against *Escherichia coli* (Ferran et al. unpublished) - Killing curves analysis

  ![Marbofloxacin](image3)

  - 10^5
  - 10^9

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Inoculum size and *in vitro* antimicrobial activity

**Low inoculum**

**High inoculum**

**Marbofloxacin concentrations** (MIC multiple)

**E. coli**

**Pasteurella multocida**

Pharmacokinetic/pharmacodynamic assessment of the effects of parenteral administration of a fluoroquinolone on the intestinal microbiota: Comparison of bactericidal activity at the gut versus the systemic level in a pig model

Aude A. Ferran⁵, Delphine Bibbal⁵, Terence Pellet⁵, Michel Laurentie⁵, Mireille Gicquel-Bruneau⁵, Pascal Sanders⁵, Marc Schneider⁶, Pierre-Louis Toutain⁵, Alain Bousquet-Melou⁵, Alain Bousquet-Melou⁵, Alain Bousquet-Melou⁵


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Inoculum size and \textit{in vitro} antimicrobial activity

Active marbofloxacin concentrations against a low inoculum have no activity against a high inoculum.
Inoculum size and *in vitro* antimicrobial activity

- Amoxicillin and Cefquinome (C4G) against *Pasteurella multocida* (Vasseur et al. unpublished) - Killing curves analysis
Inoculum size influences antimicrobial activity

1. Clinical and microbiological cure?
   1. *In vitro* evidences of the effect of inoculum size on antimicrobial activity
   2. *In vivo* evidences

2. Resistance selection/prevention at the infection site?
Inoculum size and clinical or microbiological cure

- Fluoroquinolones and beta-lactams against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Mizunaga et al. JAC 2005)
- Intraperitoneal infection in mice
- Doses associated with survival

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>S. aureus ED$_{50}$ (95% CL)$^a$</th>
<th>P. aeruginosa ED$_{50}$ (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$1 \times 10^7$ (A)</td>
<td>$1 \times 10^9$ (B)</td>
</tr>
<tr>
<td>Pazufloxacin</td>
<td>0.0253 (0.0205–0.0340)</td>
<td>0.710 (0.607–0.833)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.0325 (0.0272–0.0435)</td>
<td>0.637 (0.541–0.761)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.000933 (0.000595–0.00139)</td>
<td>0.909 (0.575–1.28)</td>
</tr>
<tr>
<td>Panipenem</td>
<td>0.00119 (0.000954–0.00139)</td>
<td>1.05 (0.602–1.65)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.0260 (0.0199–0.0330)</td>
<td>&gt;4 NC</td>
</tr>
</tbody>
</table>

Efficacious doses are 10- to 100-fold lower against a 100-fold lower inoculum
Inoculum size and clinical or microbiological cure

- **Fluoroquinolone against *Pseudomonas aeruginosa*** (Jumbe et al. JCI 2003)
- Thigh infection in mice
- Doses associated with log10 CFU reduction

**Low**

- Marbofloxacin dose: 31 mg/kg

**High**

- Marbofloxacin dose: 180 mg/kg

Kesteman et al. AAC 2009
Inoculum size influences antimicrobial activity

1. Clinical and microbiological cure?
   1. *In vitro* evidences of the effect of inoculum size on antimicrobial activity
   2. *In vivo* evidences

2. Resistance selection/prevention at the infection site?
Inoculum size and resistant mutant selection

Percentages of rats with resistant* *K. pneumoniae* in their lungs 96h after the start of marbofloxacin treatment

* Growth in the presence of half MPC

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Inoculum-size adjusted doses at different phases of spontaneously developing infections?
Inoculum-size adjusted doses for early or later treatments

**Study 1 - Fluoroquinolone**

Research article

Impact of early versus later fluoroquinolone treatment on the clinical; microbiological and resistance outcomes in a mouse-lung model of *Pasteurella multocida* infection

Aude A. Ferran, Pierre-Louis Toutain, Alain Bousquet-Mélou *
Progression of the infection

**Intratracheal inoculation**
1000 CFU/lung
*Pasteurella multocida*

**Marbofloxacin**
- **Two fixed times:** pre-patent phase and patent phase
- **Two doses:** 1 mg/kg and 40 mg/kg

**The methodology (1)**

- Early Administration: no clinical signs of infection
- Late Administration: anorexia, lethargy, dehydration

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Bacteria counts per lung (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$10^0$</td>
</tr>
<tr>
<td>10</td>
<td>$10^4$</td>
</tr>
<tr>
<td>20</td>
<td>$10^8$</td>
</tr>
<tr>
<td>30</td>
<td>$10^{10}$</td>
</tr>
<tr>
<td>40 - 50</td>
<td>$10^{10}$</td>
</tr>
</tbody>
</table>

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The results – 1. Clinical outcome (survival)

Observations: 38 hours after marbofloxacin administration or 48 hours after infection for the control group and the "early group".
The results – 1. Clinical outcome (survival)

Observations: 38 hours after marbofloxacin administration or 48 hours after infection for the control group and the « early group ».
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The results – 3. Selection of resistant bacteria

Marbofloxacin administrations

Early  Late  Early  Late

Percentages of alive mice with resistant bacteria

control  1 mg/kg  40 mg/kg

Marbofloxacin doses

No resistant bacteria

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The results – 3. Selection of resistant bacteria

Marbofloxacin administrations

Early  Late  Early  Late

Percentages of alive mice with resistant bacteria

control

1 mg/kg

40 mg/kg

Marbofloxacin doses

No resistant bacteria

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Inoculum-size adjusted doses for early or later treatments

Study 1 - Fluoroquinolone

- The lower dose of 1 mg/kg marbofloxacin during the pre-patent phase of the infection was associated to:
  - more frequent clinical cure
  - similar bacteriological cure
  - similar selection of resistant bacteria

Than the higher dose of 40 mg/kg during the pre-patent phase of the infection
Inoculum-size adjusted doses for early or later treatments

Study 2 – Beta-lactams
Progression of the infection

The methodology (1)

Air-borne contamination
10 000 CFU/lung
*Pasteurella multocida*

Amoxicillin *MIC = 0.125 µg/mL*
Cefquinome *MIC = 0.016 µg/mL*

Early Treatment
Late Treatment
mice observed twice-daily

Time after challenge (hours)

Bacterial counts per lung (Log CFU/lung)

no clinical sign of infection
anorexia, lethargy, dehydration

healthy
sick
dead
The methodology (2)

- High-inoculum adjusted doses for **sick animals**:
  - PK/PD: $T_{>\text{MIC}} > 50\%$ dosage interval

<table>
<thead>
<tr>
<th>Antibiotic concentration (µg/mL)</th>
<th>Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>4</td>
</tr>
<tr>
<td>0.01</td>
<td>8</td>
</tr>
<tr>
<td>0.1</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
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<tr>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>0.1</td>
<td>16</td>
</tr>
<tr>
<td>0.01</td>
<td>20</td>
</tr>
<tr>
<td>0.001</td>
<td>24</td>
</tr>
</tbody>
</table>

**Doses (mg/kg)**

<table>
<thead>
<tr>
<th></th>
<th>Amoxicillin</th>
<th>Cefquinome</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-inoculum adjusted doses</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Half HIAD</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>
The methodology (3)

- Low-inoculum adjusted doses for early treatments:
  - Activities against low vs high *P. multocida* inocula: *in vitro* killing curves

![Graph showing killing curves for Amoxicillin and Cefquinome against *P. multocida* at two different inoculum levels.](image)

### Doses (mg/kg)

- **Low-inoculum adjusted doses**
  - Half LIAD

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>5</td>
</tr>
<tr>
<td>Cefquinome</td>
<td>1</td>
</tr>
</tbody>
</table>

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The results – 1. Clinical cure

**Later treatments of sick animals**

% of sick mice with no symptom at day 7

<table>
<thead>
<tr>
<th>Doses mg/kg</th>
<th>AMOX</th>
<th>CEFQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>* 0%</td>
<td>* 0%</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All mice died within 7 days

**Early treatments of all animals**

% of mice with no symptom between day0 and day7

<table>
<thead>
<tr>
<th>Doses mg/kg</th>
<th>AMOX</th>
<th>CEFQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
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</tbody>
</table>

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The results – 2. Microbiological cure

**Later treatments of sick animals**

% of treated mice with bacteriological cure

<table>
<thead>
<tr>
<th>Doses mg/kg</th>
<th>AMOX</th>
<th>CEFQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>* 0 %</td>
<td>* 0 %</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
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<td>25</td>
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**Early treatments of all animals**

% of mice with bacteriological cure

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<tr>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>* 0 %</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>* 0 %</td>
</tr>
</tbody>
</table>

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Inoculum-size adjusted doses for early or later treatments

**Study 2 – Beta-lactams**

- For both amoxicillin and cefquinome, the low-inoculum adjusted doses (10-fold lower) given during the pre-patent phase of the infection were associated to:
  - Similar clinical performance (100%)
  - More frequent bacteriological cure

When compared to the high-inoculum adjusted doses (10-fold higher) given later during the patent phase of the infection (sick animals)
What impact of digestive bacterial flora?
Intratracheal inoculation

10^5 or 10^9 CFU/lung

*Pasteurella multocida*

Gnotobiotic rats

Gut colonization

Pig faeces

CTX-M (ESBL) producing *E coli*
The results – 1. Clinical cure

- 100% of infected (10^5 or 10^9 CFU) and untreated mice became sick and died

- Clinical and microbiological cure rates of 100% with:
  - High-inoculum adjusted dose (50 mg/kg) during the patent phase of the infection (sick mice)
  - Low-inoculum adjusted dose (5 mg/kg) during the pre-patent phase of the infection
The results – 2. Impact on digestive flora

- The Low-inoculum adjusted dose (5 mg/kg) during the pre-patent phase of the infection
  - Cured the pulmonary infection
  - Averted any amplification of CTX-M-producing enterobacteria
Conclusion

• Hit HARD and FAST, and stop EARLY

• For food-producing animals
  • Low-inoculum adjusted doses during the pre-patent phase of an acute infection might constitute a promising strategy for the optimization of antibiotic dosage regimens
  • To ensure infectious diseases control while minimizing the animal reservoirs of resistance genes of human concern