Antimicrobial Susceptibility Testing (AST): establishing clinical breakpoint CLSI vs. VETCAST/EUCAST

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National veterinary School of Toulouse, France
Wuhan 12/10/2015
Purpose of AST
(Antimicrobial Susceptibility Testing)

• To guide the clinical selection of an effective therapeutic intervention for the treatment of microbial infections based upon the results of in vitro susceptibility tests (AST) (the word antibiogram is also use).
The disc technic
Growth around the disc

- Complete inhibition
- Delayed growth
- Partial inhibition
- Stimulated growth

AGAR MEDIUM

Large diameter of inhibition = susceptible = S
Small diameter of inhibition = resistant = R
Clinically susceptible(s):
EUCAST Definitions

• A micro-organism is defined as susceptible by a level of activity associated with a high likelihood of therapeutic success
How to decide R and S?
Glossary

• **Breakpoint (BP) (clinical)**
  – A numerical value for S or I or R
  – A decision (*risk management*) taking into account CO values plus other considerations (harmonization...)

• **Cutoffs (CO)**
  – Numerical values to decide of the BP
    • Epidemiological or microbiological CO (CO\textsubscript{WT})
    • PK/PD CO (CO\textsubscript{PD})
    • Clinical CO (CO\textsubscript{CL})
  – CO are computed (*risk assessment*)
Two international organizations

- Located in US but not a US committee
- An EU Committee
EUCAST
(see their website)

VetCAST - the veterinary committee on antimicrobial susceptibility testing

VetCAST is a EUCAST subcommittee dealing with all aspects of antimicrobial susceptibility testing of bacterial pathogens of animal origin and animal bacteria with zoonotic potential. The subcommittee will operate within the format and structure of EUCAST (The European Committee on Antimicrobial Susceptibility Testing).

Read more about ➔ VetCAST

<- Back to: EUCAST News
Remits of EUCAST

1. To harmonise clinical breakpoints for existing antimicrobial agents in Europe,
2. To determine clinical breakpoints for new agents,
3. To set epidemiological (microbiological) breakpoints (cut-off),
4. To revise breakpoints as required,
5. To harmonise methodology for antimicrobial susceptibility testing,
6. To develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms
7. To liaise with European governmental agencies and European networks involved with antimicrobial resistance and resistance surveillance.
VETCAST, a subcommittee of EUCAST

VetCAST is a EUCAST subcommittee dealing with all aspects of antimicrobial susceptibility testing of bacterial pathogens of animal origin and animal bacteria with zoonotic potential. The subcommittee will operate within the format and structure of EUCAST (The European Committee on Antimicrobial Susceptibility Testing).

- VetCAST vision, strategy, remits, Steering committee and members.
- The VetCAST was formed on the 27 of April 2015, in Copenhagen during ECCMID 2015.

- Kickoff meeting - presentation by D Mevius
- Available breakpoints of antimicrobials for veterinary use - presentation by K Veldmann
- Setting clinical breakpoints - methodological aspects - presentation by P-L Toutain.
- The Minutes of the first closed and open VetCAST subcommittee meetings (Copenhagen 27 April, 2015).
What is VETCAST?

• VETCAST is a EUCAST subcommittee
  – It deals with all aspects of antimicrobial susceptibility testing (AST) of bacterial pathogens of animal origin and animal bacteria with zoonotic potential.
  – The subcommittee will operate within the format and structure of EUCAST (The European Committee on Antimicrobial Susceptibility Testing).
The 2 main objectives of VetCAST

1. To determine antimicrobial breakpoints specific to the veterinary field;
   – Currently, absence of animal species-specific breakpoints
2. To harmonize veterinary antimicrobial susceptibility testing in the European Union (EU);
VETCAST for EMA

- To provide advice on the type and quality of the MIC, pharmacokinetic (PK) and clinical data needed for setting clinical breakpoints
- To define clinical MIC breakpoints for new veterinary antimicrobial agents
- To revise breakpoints for generic drugs
- To advice on the bacterial spectrum of veterinary antimicrobial agents
CLSI/VAST
(NCCLS)
CLSI: VAST

• The CLSI Veterinary Antimicrobial Susceptibility Testing (VAST) committee is in charge in defining clinical breakpoints for new and generic veterinary antimicrobial agents.
CLSI ‘s VAST

About CLSI’s Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST)

Volunteers for the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) collaborate to develop standards and guidelines that promote accurate antimicrobial susceptibility testing and appropriate reporting.
CLSI/VAST: members

• **Individuals** from the following types of areas join together to pool knowledge, expertise, and viewpoints:
  – Veterinary microbiology laboratories
  – Government agencies (FDA)
  – Veterinary care providers and educators
  – Pharmaceutical and diagnostic microbiology laboratories
CLSI released many documents explaining how they proceed.

**VET01-S2**
Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Second Informational Supplement

**VET01-A4**
Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Fourth Edition

Add to your basket (not free of charge as for EUCAST)
CLSI/VAST approved breakpoints for label applications in food animals

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Disease/Pathogen(s)</th>
<th>Zone (mm)</th>
<th>Concentrations (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>Bovine respiratory disease - Mannheimia haemolytica, Pasteurella multocida, Histophilus somni</td>
<td>≥21</td>
<td>18-21</td>
</tr>
<tr>
<td></td>
<td>Swine respiratory disease - Actinobacillus pleuropneumoniae, Pasteurella multocida, Salmonella choleraesuis, Streptococcus suis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Bovine mastitis - Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli</td>
<td>≥21</td>
<td>18-21</td>
</tr>
<tr>
<td>Ceftriaxone (intramammary)</td>
<td>Bovine mastitis - Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>Bovine respiratory disease - Mannheimia haemolytica, Pasteurella multocida, Histophilus somni</td>
<td>≥21</td>
<td>17-20</td>
</tr>
<tr>
<td>Flurophenicol</td>
<td>Bovine respiratory disease - Mannheimia haemolytica, Pasteurella multocida, Histophilus somni</td>
<td>≥19</td>
<td>15-18</td>
</tr>
<tr>
<td></td>
<td>Swine respiratory disease - Actinobacillus pleuropneumoniae, Pasteurella multocida, Streptococcus suis Type 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flurophenicol</td>
<td>Swine respiratory disease - Salmonella choleraesuis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin/ Novobiocin</td>
<td>Bovine mastitis - Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberiens</td>
<td>≥18</td>
<td>15-17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pirimycin</td>
<td>Bovine mastitis - Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberiens</td>
<td>≥13</td>
<td>11-13</td>
</tr>
<tr>
<td>Spectinomycin sulfate</td>
<td>Bovine respiratory disease - Mannheimia haemolytica, Pasteurella multocida, Histophilus somni</td>
<td>≥14</td>
<td>11-13</td>
</tr>
<tr>
<td></td>
<td>Swine respiratory disease - Actinobacillus pleuropneumoniae</td>
<td>≥9</td>
<td>10</td>
</tr>
<tr>
<td>Tiamulin</td>
<td>Bovine respiratory disease - Mannheimia haemolytica</td>
<td>≥14</td>
<td>11-13</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>Bovine respiratory disease - Mannheimia haemolytica</td>
<td>≥14</td>
<td>11-13</td>
</tr>
<tr>
<td></td>
<td>Swine respiratory disease - Pasteurella multocida, Actinobacillus pleuropneumoniae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A given animal species
A given disease
2 given pathogens
Setting clinical breakpoint
Methodological aspects
Glossary: risk analysis

Diameters \((S,R)\) \hspace{2cm} \text{Risk communication}

\text{Breakpoint} \hspace{2cm} \text{Risk management}

\text{Cut-off} \hspace{2cm} \text{Risk assessment}
Development of a (clinical) Breakpoint (BP)

- \( \text{CO}_{\text{WT}} \)
- ECOFFs
- \( \text{CO}_{\text{PK/PD}} \)
- \( \text{CO}_{\text{CL}} \)

Diameter

Clinical BP
Epidemiological cut-off values
(\text{CO}_{\text{WT}}, \text{ECOFFs})
Epidemiological cut-off values (ECOFFs)

• ECOFFs (or microbiological breakpoints) are related to the distribution of MICs of wild type organisms lacking acquired or mutational resistance to the antimicrobial agent in question.

• The ECOFF is essentially the upper MIC value of the wild type distribution.
ECOFFs: SOP

- ECOFFs are defined for relevant species according to procedures detailed in the introduction to the MIC and zone diameter distributions website and in EUCAST SOP 7.0 (in preparation).
The epidemiological cutoff: ECOFF/COWT

The case of unimodal distribution

e.g., MIC distributions for tulathromycin with 728 strains of *M. haemolytica* isolated from bovine respiratory disease. Note the “wild-type” population with MIC values \( \leq 8 \mu g/ml \).
The epidemiological cutoff: ECOFF/CO\textsubscript{WT}

Bimodal distribution

![Graph showing bimodal distribution with Wild-Type and Non-wild-Type categories.](image)

(Cutoff CLSI)
Ciprofloxacin & Acinetobacter baumannii

MIC
Epidemiological cut-off (ECOFF): 1 mg/L
Wildtype (WT) organisms: ≤ 1 mg/L

4211 observations (115 data sources)
Statistical method for ECOFF/CO_{WT}

ORIGINAL ARTICLE

Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values

J. Turnidge¹, G. Kahlmeter² and G. Kronvall³

RangeFinder and ECOFFinder

Normalized Resistance Interpretation as a Tool for Establishing Epidemiological MIC Susceptibility Breakpoints

Göran Kronvall*
Why no to split a wild population
<table>
<thead>
<tr>
<th>MIC-data available tetra/doxycycline</th>
<th>EUCAST</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactobacillus reuteri</strong></td>
<td>0.002 0.004 0.008 0.016 0.032 0.064 0.125 0.25 0.5 1 2 4 8 16 32 64 128 256 512</td>
</tr>
<tr>
<td><strong>Lactobacillus rhamnosus</strong></td>
<td>0 0 0 0 0 0 0 0 1 7 19 26 1 5 31 22</td>
</tr>
<tr>
<td><strong>Lactobacillus sakei</strong></td>
<td>0 0 0 0 0 0 0 0 4 27 29 16 3 1 0 3</td>
</tr>
<tr>
<td><strong>Lactococcus lactis</strong></td>
<td>0 0 0 0 0 0 1 48 23 5 1 0 0 0 0 3 2 6</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes</strong></td>
<td>0 0 0 0 0 0 2 6 20 26 12 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td><strong>Mannheimia haemolytica</strong></td>
<td>0 0 0 0 0 0 0 3 29 23 1 1 2 16 38 11 4</td>
</tr>
<tr>
<td><strong>Moraxella catarrhalis</strong></td>
<td>0 0 0 0 0 2 172 3888 3357 807 256 114 36 20 26 1 1 0</td>
</tr>
<tr>
<td><strong>Morganella morgani</strong></td>
<td>0 0 0 0 0 0 6 40 31 18 5 4 23 15 25 3 0</td>
</tr>
<tr>
<td><strong>Neisseria gonorrhoeae</strong></td>
<td>0 0 0 0 0 0 0 112 392 497 932 1172 1314 366 116 270 298 93 37 6 3</td>
</tr>
<tr>
<td><strong>Neisseria meningitidis</strong></td>
<td>0 0 0 0 0 0 0 1 27 28 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td><strong>Pasteurella multocida</strong></td>
<td>0 0 0 0 0 0 3 19 111 54 44 18 9 17 40 13 7 1 0</td>
</tr>
<tr>
<td><strong>Proteus mirabilis</strong></td>
<td>0 0 0 0 0 0 0 0 0 1 7 4 2 5 61 715 172 28 10</td>
</tr>
<tr>
<td><strong>Proteus spp</strong></td>
<td>0 0 0 0 0 0 0 3 13 3 2 8 4 2 0</td>
</tr>
<tr>
<td><strong>Proteus vulgaris</strong></td>
<td>0 0 0 0 0 0 0 1 1 2 7 0 3 4 0</td>
</tr>
</tbody>
</table>
For more details

Kahlmeter - ECOFFs, ECOFFs, ECOFFs

ECOFFs
ECOFFs
ECOFFs
ECOFFs

MIC wild type distributions and epidemiological cut-off values

Gunnar Kahlmeter
EUCAST, ESCMID and ECDC
Clinical microbiology, Växjö, Sweden

EUCAST workshop, ECOMID 2013
The setting of a PK/PD cutoff
The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach

The setting of a PK/PD CO

Step 1
Selection of a PK/PD index predictive of clinical efficacy and/or prevention of resistance

Step 2
Determination of the critical value (size) of the selected PK/PD index

Step 3
Computation, for a given animal species and for all possible (not probable) MICs of the percentage (proportion) of animals able to achieve the critical value of the selected PK/PD index (computation of so-called Target Attainment Rates (or TAR))
Step 1: Selection of a PK/PD index

Rem: it was shown that for all drugs/formulations having a long half-live that AUC/MIC is the appropriate index (AU/MIC: universal index?)
Step 2: Determination of the critical value (size) of the selected PK/PD index
A-Clinical determination of the numerical target value for the $\text{CO}_{\text{PK/PD}}$
Logistic regression analysis

**FIG. 1.** Cure rate after treatment with fluconazole in patients ($n = 132$) with oropharyngeal candidiasis. From these patients, the MIC distribution of the *Candida albicans* strains causing the infection was determined. Patients received different doses of fluconazole and the area under the curve (AUC) was estimated in each patient. There were seven different fAUC/MIC ratio values, resulting in seven groups. The proportion of patients cured in each group was plotted against the AUC/MIC ratio and the relationship was determined using the $E_{\max}$ model. The figure clearly shows the proportionality between fAUC and cure rate, whereas the MIC is inversely proportional to the cure rate [13].
B-Preclinical determination of the numerical target value for the $\text{CO}_{\text{PK/PD}}$
Preclinical determination of CO\textsubscript{PK/PD}

• **In vivo: (target) animal models**
  – mice that are rendered neutropenic and infected with an inoculum of \(10^6\) CFU/mL of microorganisms in the thigh or lung.
  – Treatment is then initiated and after 24 or 48 h the total bacterial count is determined for each organ.
  – Using different doses and dosing intervals, ranges of exposure are obtained and are subsequently plotted against the number of CFU to establish exposure–response relationships.

• **in vitro: killing curves & hollow fibres infection models**
How were established these indices?

1-Cyclophosphamide
2-Pathogen challenge
3-Antibiotic

4-PK parameters
AUC, Cmax, T>MIC

4-PD Endpoints:
• CFU
• Mortality rate

Vega et al. 37th ICAAC 1997
R. Garraffo Nov 2003
Relationship between AUC/MIC and % of death for a FQ against a G-
Preclinical determination of CO\textsubscript{PK/PD}

- Preclinical
  - In vitro
    - Static
      - Killing curves
    - Dynamic
      - Hollow fibers
      - Others
The hollow fiber
Step 1
Selection of a PK/PD index predictive of clinical efficacy and/or prevention of resistance

Step 2
Determination of the critical value (size) of the selected PK/PD index

Step 3
Computation, for a given animal species and for all possible (not probable) MICs, of the percentage (proportion) of animals able to achieve the critical value of the selected PK/PD index (computation of so-called Target Attainment Rates (or TAR))
Step 3: Computation of the TAR (%) for the critical value of the selected index for the different possible MICs (TAR are stratified by MIC)

• More literally the question is:
  – E.g. What is the critical (maximal) MIC for which we can guarantee that plasma drug concentration will be:
    • above a possible MIC for X% of the dosage interval (often 50 or 80%) for T>MIC
    • or≥ to a given AUC/MIC
  – for the intended dosage regimen in at least 90% of subjects of the targeted population
Step 3: Computation of the TAR (%) for the critical value of the selected index for the different possible MICs (TAR are stratified by MIC)

1. PK raw data
2. Population modeling
3. Monte Carlo Simulation (n=5000 animals)
4. TAR(%)
See next presentation

Use of Monte Carlo simulation to determine pharmacodynamic cutoffs of amoxicillin to establish a breakpoint for antimicrobial susceptibility testing in pigs

Julien F. Rey, DVM; Céline M. Laffont, PhD; Siska Croubels, Pharm D; Patrick De Backer, DVM; Claudine Zemirline, DVM; Eric Bousquet, DVM; Jérôme Guyonnet; Aude A. Ferran, DVM; Alain Bousquet-Melou, DVM; Pierre-Louis Toutain, DVM
Three case of marbofloxacin in the horse
Population investigation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Montréal</th>
<th>Toulouse1</th>
<th>Utrecht</th>
<th>Vienne</th>
<th>Toulouse2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population (n=131)</td>
<td>44</td>
<td>58</td>
<td>9</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female , (n=68)</td>
<td>31</td>
<td>23</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>gelding (n=48)</td>
<td>11</td>
<td>25</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Male (n=13)</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>healthy(n=60)</td>
<td>21</td>
<td>18</td>
<td>9</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>hospital (n=70)</td>
<td>23</td>
<td>40</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Age (Yrs) (n=97)</td>
<td>9.9</td>
<td>10.5</td>
<td>12.9</td>
<td>8.1</td>
<td>15.8</td>
</tr>
<tr>
<td>BW(Kg) (n=127)</td>
<td>482</td>
<td>397</td>
<td>596</td>
<td>401</td>
<td>564</td>
</tr>
</tbody>
</table>
Marbofloxacin

PRED (population predicted concentration) vs observed concentrations (µg/mL). Data are evenly distributed about the line of identity, indicating an appropriate structural model could be found for most individuals.

IPRED (Individual predicted concentrations) vs. observed concentrations (µg/mL). IPRED were obtained by setting random effects to the 'post hoc' or empirical Bayesian estimate of the random effects for the individual from which the DV observation was made.
# Marbofloxacin: CO\textsubscript{PK/PD}

<table>
<thead>
<tr>
<th>MIC</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>125</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03125</td>
<td>100</td>
<td>100</td>
<td>99.8</td>
<td>98.8</td>
<td>95.6</td>
<td>58.6</td>
</tr>
<tr>
<td>0.0625</td>
<td>100</td>
<td>98.8</td>
<td>92.3</td>
<td>78.2</td>
<td>58.6</td>
<td>8.4</td>
</tr>
<tr>
<td>0.125</td>
<td>98.8</td>
<td>78.2</td>
<td>44.9</td>
<td>21.1</td>
<td>8.4</td>
<td>0.051</td>
</tr>
<tr>
<td>0.25</td>
<td>78.2</td>
<td>21.1</td>
<td>4.2</td>
<td>0.712</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>21.1</td>
<td>0.712</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>1</td>
<td>0.712</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

This table provides the percentage of horses able to achieve AUC/MIC of 24, 48...250h for MIC ranging from 0.03125 to 2µg/mL.
Assessment of the currently used dosage regimen

The goal is for the committee to have an opinion on the validity of the current dosage regimen (especially for old drugs) to discuss the value of its AST, to manage the situation where several different dosage regimen are existing in the EU, to prioritise bugs for which we need urgently an AST ...

Required information: a model to compute the dose and ad hoc parameters (PK) and data sets (PD) for the Monte Carlo Simulations

PK
(the same as for step 3) to generate population parameters

PD: MIC distributions
(the same as for the establishment of the ECOFF
## MIC Marbofloxacin

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC values (µg/mL)</th>
<th>MIC50 (µg/mL)</th>
<th>MIC90 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.004</td>
<td>0.008</td>
<td>0.015</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Pantoea agglomerans (Enterobacter agglomerans)</td>
<td>37</td>
<td>3</td>
<td>0.022</td>
</tr>
<tr>
<td>Rhodococcus equi</td>
<td>3</td>
<td>17</td>
<td>0.665</td>
</tr>
<tr>
<td>Streptococcus zooepidemicus</td>
<td>29</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>
Computation of the dose

\[
Dose = \frac{Clearance \ (\text{per hours}) \times \left( \frac{AUC}{MIC} \right)_{BP} \times MIC}{F\% \times fu}
\]

Log normal distribution of CL/F (pop PK)

Observed MIC distributions

BP 125h
Monte Carlo simulation: applied to PK/PD models

Generate random AUC and MIC values across the AUC & MIC distributions that conforms to their probabilities

Calculate a large number of AUC/MIC ratios

Plot results in a probability chart

Model: AUC/MIC

PDF of AUC

PDF of MIC

PDF of AUC/MIC

% target attainment

Marbofloxacin
Dose for Gram negative

lower than the Marketed dose: 2mg/kg
Marbofloxacin
Dose for Gram positive

much higher than the marketed dose of 2mg/kg
Conclusions for marbofloxacine in horses

• The current dose is able to cover 100% of horses for gram negative pathogens
  – (rather seldom in horses)
• The current dose is unable to treat a gram positive infection in horses

• Thus no AST for horses is required
Clinical cutoff
The clinical CO (CO_{CL})

The CO_{CL} is based upon the collection of isolates obtained during the clinical effectiveness studies. CO_{CL} reflects the upper limit of the MIC values associated with a high likelihood of clinical success [probability of cure (POC)].

There is no set method for establishing the CO_{CL}, and no hard target for POC.
Probability of cure (POC)

- Logistic regression can be used to link MIC values (as independent variable) to the probability of a clinical success.

$$POC = \frac{1}{1 + e^{-a + bf(MIC)}}$$

- Dependent variable
- Ceiling response
- Sensitivity
- Independent variable (here collected MICs during clinical trials) + other covariables

2 parameters: $a$ (ceiling effect) & $b$ (slope of the MIC-effect curve)
Logistic modelling: difficulties

• Scarse an unbalanced data (not enough failure)
• Possible international project of research?
Example of clinical *cutoff* higher than the epidemiological CO

**Fig. 1.** Ciprofloxacin MIC distribution of *Escherichia coli* isolates (http://www.eucaast.org). Epidemiological cut-off (ECOFF) values and clinical susceptible (S) and resistant (R) breakpoints from CLSI and EUCAST committees are indicated. The clinically susceptible population (below the clinical susceptible breakpoint) includes part of the microbiologically resistant population (low-level resistant bacteria, presumably expressing *qnr*-like genes or other PMQR mechanisms or first step *gyrA* mutations) and the wild-type population (below the ECOFF value and presumably without resistance mechanisms). The clinically resistant population (beyond the clinical resistant breakpoint) includes isolates with high-level resistance mechanisms (most probably double-step *gyrA* mutants or a combination of *gyrA* with *parC* mutations).
Clinical breakpoint
Setting the breakpoint

• A risk management exercise
• Not a scientific exercise but should be scientifically acceptable
• CO and others consideration
  – Harmonization (e.g., different dosage regimen across EU, no splitting of the wild distribution...
Decision tree to select a BP from the three CO

(1) “S” is based on COWT and COCL

COWT ≠ COCL

When COWT ≠ COCL, the CO_PD is used as a weighting factor for the final determination of “S.”

COCL > COWT

COCL > CO_PD > COWT

CO_PD > COCL > COWT

(2) “S” = COCL

COCL > COWT > CO_PD

(3) “S” = COWT

COWT > CO_PD > COCL

CO_PD > COWT > COCL

(4) “S” = COWT

COWT > COCL > CO_PD

(5) “S” = COCL

Figure C2. Susceptibility Breakpoint (SBPT) Decision Tree
Clinical breakpoint

• CLSI: vote
• EUCAST: consensus
Conclusion

• Veterinary medicine needs specific breakpoints
• International cooperation is possible to settle the epidemiological cutoff
• Currently, no well established approach to select a clinical cutoff
• The value of AST has to be supported by computing their predictive value and diagnostic gains