A Clinical Pharmacologists View of the Interaction of Antimicrobials and Bacteria in Food Animals

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Human antibiotics are fed routinely to livestock. And it’s making human diseases harder to cure.

SaveAntibiotics.org

The PEW Campaign on Human Health and Industrial Farming
Bacterial populations exposed to antimicrobials on farm

Selection for resistant organisms on farm

Increased incidence of resistant organisms on farm

Transfer through the food chain or direct transfer

Presence of food animal derived resistant bacteria in a human

Contribution of food animal derived resistant bacteria to human disease

Treatment failure or prolonged disease course due to pathogen resistance

The Thought Process

Release

Exposure

Consequence
Sooo, if we are going to talk about release...

- We must define and evaluate
  - the antimicrobial pressure
  - the bacteria of interest
  - the interaction between the antimicrobial and bacteria of interest
  - the outcome of this interaction
  - the definition of “resistance”
  - concern about transfer of resistant bacteria or transmissible resistance elements
Classes of Antimicrobial Use in Food Animals

- We get confused as to the reason for classification
  - Therapeutic intent?
  - Probability of selection for resistant bacteria?
  - Societal justification?
Classes of Antimicrobial Use in Food Animals

- FDA/CVM approval classifications
  - Increase in rate of gain
  - Increase in feed efficiency
  - Prevention
  - Control
  - Therapy/Treatment

- Classifications by bacteria
  - They don’t care
USING TETRACYCLINES AS AN EXAMPLE
The Tetracyclines - Pharmacodynamics

- Trying to predict - Time above MIC? Or AUC/MIC?
  - There is only one paper that I have found which addresses the first generation tetracyclines (CTC, OTC, TC).
    - An E-max model for tetracycline displayed bacteriostatic activity against \textit{E. coli}. (Regoes, 2004)
  - Information on AUC/MIC, T>MIC, or Cmax:MIC is not available in the literature for the first generation tetracyclines.
  - These data are often for different organisms in culture, anyway.
Regardless of what we predict as to pharmacodynamic indices for the tetracyclines, they may or may not apply to gut activity anyway.

Even for systemic effects, treating pharmacodynamic indices as absolutes will likely lead us astray.

- i.e., what happens below the MIC?
- Where is the concentration measured?
The Tetracyclines – “S”, “I”, and “R”

- What is “resistant”? 
- Classic veterinary breakpoints adapted from human medicine are 4, 8, and 16 µg/ml for “S”, “I”, and “R”, respectively.
- These are substitution variables for in vivo activity based on the ability of the antimicrobial to inhibit growth in the laboratory.
- There are now “generic” breakpoints for swine and bovine respiratory disease.
There are extensive, transmissible resistance genetic elements out there

- e.g., a 2010 review of the tetracycline resistome notes 1,189 different reported resistance genes present in 84 bacterial genera, which included 354 bacterial species (Thaker, 2010)

- These genes comprise 41 classes, with three major mechanisms
  - Actively pumping the drug out of the cell
  - Enzymatic degradation of the drug
  - Protection of the drug binding site
The Tetracyclines – Resistance Transfer

- Chopra and Roberts (2001)
  - Gram-negative and Gram-positive genes coding for tetracycline efflux are generally associated with plasmids.
  - tet(S) and tet(O) encode for ribosomal protection and are located both in the chromosome and in conjugative plasmids
  - tet(M) and tet(Q) (also ribosomal protection) and typically associated with conjugative transposons
  - Other mechanisms include enzymatic inactivation (tet(X) and tet(37))
  - Mosaic genes have also been described, which are combinations of individual genes (e.g., tet(O/32/O))
CTC: 0.1 mg/hd per day in calves up to 250 lbs
CTC: 25-70 mg/hd per day in calves 250-400 lbs
CTC: 70 mg/hd per day in growing cattle over 400 lbs
CTC: 350 mg/hd per day in beef cattle under 700 lbs
CTC: 0.5 mg/lb per day in beef cattle over 700 lbs
CTC: 350 mg/hd per day in beef cattle
CTC: 400 g/ton to provide 10 mg/lb per day in calves up to 250 lbs
CTC: 0.5 to 2.0 g/hd per day
CTC: 25-70 mg/hd per day in calves 250-400 lbs
CTC: 70 mg/hd per day in growing cattle over 400 lbs
CTC: 0.1 mg/hd per day in calves up to 250 lbs

These are not all of the CTC, TC, and OTC indications, but are selected to illustrate the regimen range.
<table>
<thead>
<tr>
<th>Percent of total dose</th>
<th>Percent of oral dose detected as active compound in feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.2%, Crocker and Robinson, (2002), % of daily CTC dose in pig feces by biosensor, ACR</td>
</tr>
<tr>
<td>2.0</td>
<td>0.15 to 1%, van Marwyck (1958), % of daily dose recovered in daily fecal output in humans, ACR</td>
</tr>
<tr>
<td>4.0</td>
<td>0.15% to 3%, Tancrede and Barakat, (1989), unspecified assay, percentage of daily OTC dose recovered from daily fecal output of humans. ACR</td>
</tr>
<tr>
<td>6.0</td>
<td>1% to 5.2%, Perrin-Guyomard, et al. (2001) Upper end of range of detected TET percentage of daily dose in daily fecal output of HFA model mice.</td>
</tr>
<tr>
<td>10.0</td>
<td>Outlier, 67.9% of oral CTC dose detected over 48 hours in rats. Eisner and Wulf, (1963)</td>
</tr>
<tr>
<td>12.0</td>
<td>13.4%, Sweeney, et al. (1957) Percent of a single 250 mg oral TET dose detected over 72 hrs in humans.</td>
</tr>
<tr>
<td>14.0</td>
<td>ACR = Additional Calculations Required, using data from other sources for daily fecal output, feed consumption, etc.</td>
</tr>
<tr>
<td>16.0</td>
<td>1% to 5.2%, Perrin-Guyomard, et al. (2001) Upper end of range of detected TET percentage of daily dose in daily fecal output of HFA model mice.</td>
</tr>
</tbody>
</table>
Carmen, et al. (2006) evaluated three concentrations of tetracycline in a chemostat system inoculated with human fecal flora. Concentrations of 0.15, 1.5, and 15 µg/ml were used in the systems, equivalent to daily doses of 0.025, 0.25, and 2.5 mg/kg per day in a 60 kg human (based on fecal concentration data by van Marwyck, 1958). Statistical analysis identified the lowest and middle concentrations as having no observable adverse effect on the bacterial population.
Perrin-Guyomard, et al. (2001) used a human-flora-associated (HFA) mouse model to evaluate water tetracycline concentrations of 0, 1, 10, and 100 mg/liter administered for 8 weeks.

Upon further calculation, these are equivalent to doses of 0, 0.125, 1.25, and 12.5 mg/kg BW.

The authors cited the highest dose as being capable of disrupting the capability to resist Salmonella infection by a resistant isolate.

At the lowest dose, there were transient increases in percent resistant Bacteroides fragilis and Enterococci. These effects were more pronounced at higher doses.

**NOAEL for Tetracycline?**
Tancrede and Baraket (1987) administered 2, 20, or 2000 mg/day to human volunteers for 7 days.

In 60 kg humans, this would be equivalent to 0.03, 0.33, 33 mg/kg per day.

The low dose caused no change in % resistance in the dominant anaerobes.

The two high doses did induce changes in resistance.
**U. S. CTC, TC and OTC Cattle Approval Examples (Feed and Water)**

| CTC: | 0.1 mg/hd per day in calves up to 250 lbs |
| CTC: | 25 - 70 mg/hd per day in calves 250 – 400 lbs |
| CTC: | 70 mg/hd per day in growing cattle over 400 lbs |
| CTC: | 350 mg/hd per day in beef cattle under 700 lbs |
| CTC: | 0.5 mg/lb per day in beef cattle over 700 lbs |
| CTC: | 400 g/ton to provide 10 mg/lb per day in calves up to 250 lbs |
| OTC: | 0.5 to 2.0 g/hd per day |

**Feed efficiency/Rate of gain**

| CTC: | 10 mg/lb BW for up to 5 days |
| TC: | 22 mg/kg for 3-5 days in calves |
| OTC: | 0.5 to 2.0 g/hd per day |

**Prevention/Control**

| CTC: | 70 mg/hd per day in growing cattle over 400 lbs |
| CTC: | 350 mg/hd per day in beef cattle |
| TC: | 22 mg/kg for 3-5 days in calves |
| OTC: | 0.5 to 2.0 g/hd per day |

**Treatment**

| CTC: | 0.1 mg/hd per day in calves up to 250 lbs |
| CTC: | 25 - 70 mg/hd per day in calves 250 – 400 lbs |
| CTC: | 70 mg/hd per day in growing cattle over 400 lbs |
| CTC: | 350 mg/hd per day in beef cattle under 700 lbs |
| CTC: | 0.5 mg/lb per day in beef cattle over 700 lbs |
| CTC: | 400 g/ton to provide 10 mg/lb per day in calves up to 250 lbs |
| OTC: | 0.5 to 2.0 g/hd per day |

These are not all of the CTC, TC, and OTC indications, but are selected to illustrate the regimen range.

Cattle have a markedly different intestinal tract structure compared to the model species.
All models are wrong, some are just useful

These studies are not presented as predicting NOAELs in food animals, however…

They do display a consistent dose-effect relationship, with higher doses having a greater effect on fecal flora during the same dosing interval.

Changes from the lower doses were often shown to be transient, even for prolonged administration.
TETRACYCLINES – WHAT ACTUALLY HAPPENS?
CTC in Feed

- CTC at 22 mg/kg BW in feed for days 0 through 4, 6 through 10 and 12 through 16.
- Fecal samples on days -7, 0, 2, 6, 8, 12, 14, 19, 22, 26, and 33.
- Resistance to CTC in *E. coli* and *Enterococcus* was monitored.
- Exposure to CTC was associated with a significant temporary increase in log2 MIC for both genera, but returned to pre-exposure values by day 33.
All ceftiofur resistant E. coli isolates were also resistant to tetracycline, but...

Exposure to chlortetracycline led to a significant decrease in the proportion of E. coli resistant to ceftiofur during exposure.

Tetracycline Resistance

- A function of concentration and time
  - Goes to baseline when drug cleared from system
  - Regardless of whether animal, human, or *in vitro*

Tetracycline Administration

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Slide courtesy Dr. Guy Loneragan
“Subtherapeutics” and E. coli

- 300 crossbred steers on 6 treatments. (5 pens of 10 each treatment). Label inclusion rates.
  - Control
  - Chlortetracycline/sulfamethazine 44 ppm each (Aureo S-700)
  - Chlortetracycline 11 ppm (Aureomycin)
  - Monensin 25 ppm (Rumensin)
  - Tylosin 11 ppm (Tylan)
  - Virginiamycin 31 ppm (V-Max)
“Subtherapeutics” and E. coli

- Silage-based diet for first 115 days, adapted to a barley-based diet over 21 days and then fed for an additional 179 days.
- The treatments were administered starting at 17 days and included for 61 days in the silage diet, then discontinued for 86 days.
- The treatments were reintroduced for a period of 42 days during the grain based diet.
“Subtherapeutics” and E. coli

- In-weights of 198 ± 20 kg
- Figure a 1 kg/day gain during the 115 day backgrounding period (end weight 313 kg, average weight for period of 255 kg)
- For the feeding period, figure a 1.6 kg/day gain for the 200 day period, for a final weight of 575 kg (1265 lbs). The medicated feed was fed from days 51 to 93 of the finishing period, for an estimated average weight during the administration period of 428 kg.
“Subtherapeutics” and E. coli

- Cattle were consuming about 7.8 kg/day (DMB) during backgrounding period (silage) then about 11.0 kg/day during finishing.
- Antimicrobial Intake would therefore be:
  - Backgrounding period (administered 61 days)
    - Chlortetracycline/sulfamethazine – 343 mg (1.4 mg/kg) each compound/day
    - Chlortetracycline – 85.8 mg (0.3 mg/kg)/day
  - Feeding period (administered 42 days)
    - Chlortetracycline/sulfamethazine – 484 mg (1.1 mg/kg) each compound/day
    - Chlortetracycline – 121 mg (0.3 mg/kg)/day
Except for control and monensin groups, the number of E. coli isolated on non-selective media were lower in the silage period.

Including tetracycline alone in the diet increased the tetracycline-resistant E. coli population from approximately 3% of isolates to 10%.

Tetracycline/sulfamethazine increased the percentage to 19.5%.

- And also increased the percentage of ampicillin-resistant E. coli isolates.
“Subtherapeutics” and E. coli

- Removing the treatments from the diets for 56 days during the silage period and 40 days during the grain period did not significantly alter the prevalence of cattle shedding tetracycline- or ampicillin-resistant E. coli.

Diversity and Distribution of E. coli administered “subtherapeutics”

- 197 day study administering either CTC (350 mg/hd per day) or CTC/sulfamethazine (same rate each per day)
- “…E. coli from day 0 showed diverse antibiogram profiles and strain types, which by the finishing phase were limited to up to three, irrespective of the treatment.”
- “…an increased linked inheritance of ampicillin and tetracycline resistance genes and prevalence of specific strains at day 197.”
Diversity and Distribution of E. coli administered “subtherapeutics”

FIG. 2. Ampr (A) and Tetr (B) E. coli counts (log CFU g⁻¹ [wet weight]) in periods A and H with no antibiotic treatment (control), 350 mg head/day chlortetracycline (T), and 350 mg head/day each chlortetracycline and sulfamethazine (TS).

Very complicated, but we do cause changes in enteric populations with oral antimicrobial use.

A definite dose-response relationship demonstrated in some studies.

In some studies, the changes were transient in at least some of the categories.

If we lop off the most politically acceptable category to “cut down use”, then we end up with a precedent of the precautionary principle for addressing the much more important, and in my mind the more likely to have an effect, prevention and control claims.
Let’s not become fixated on the red light!

- We also have developing issues of resistance in certain classes of food animal pathogens.
  - *Salmonella newport*
  - *Mannheimia haemolytica*
  - *Pasteurella multocida*
The example of the tetracyclines illustrates the multifaceted interaction between antimicrobials and enteric organisms as well as food animal pathogens.

In relation to antimicrobial resistance regulation and legislation, antimicrobial use classification as “subtherapeutic” or “therapeutic” across all antimicrobials is about societal justification, not about potential for resistance selection in enteric bacteria populations.
The relative resistance selection contribution of dose and duration is ill-defined

- In fact, the effect of duration of therapy on therapeutic outcome is ill-defined in both human and veterinary medicine
- In food animal medicine, we have multiple studies on post-treatment intervals after single injections, but very little on the effects of duration of therapy.