Heavy metals as alternatives to antibiotics: Panacea or Pandora’s Box?

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Lost in translation?

• Some topics will leave little room for misinterpretation…

• Others references to popular culture might not always so clear…

Heavy Metal
Panacea

- In Greek mythology, Panacea was the goddess of ‘universal remedy’
- In medicine it refers to a substance meant to cure all diseases
- More generally, a panacea refers to something intended to completely solve a large, multi-faceted problem

Heavy metals: general

- Vital roles as “trace elements” in biological reactions
- Used extensively in food animal production to maintain normal physiology and healthy status of animals
- **Copper**, **zinc**, and arsenic are the widely used heavy metals at levels beyond the nutritional requirement of the animals
Copper

- Required for the synthesis of hemoglobin
- Activation of oxidative enzymes necessary for metabolism of nutrients
- Maintains normal immune status of the animals
- Supplemented as sulfate, carbonate, and chloride salts
- Widely used as a growth promoter in both cattle and swine production in the US and EU

NRC, 1998 & 2000
History of Cu usage

• Copper
  – Known to stimulate growth in piglets (Bowler et al., 1955)
  – Growth-promoting action of copper is likely due to its antimicrobial properties (Fuller et al., 1960)
  – Nutritional studies to determine the growth promoting effects of copper (Bowland et al., 1961)
Zinc

- Commonly used in swine production for growth promotional activities
- Plays a role in protein, carbohydrate, and lipid metabolism
- Supplemented as oxide, sulfate, carbonate, chloride, and zinc metal dust
- Most often, used in combination with copper

NRC, 1998 & 2000
History of Zn usage

- Zinc
  - Increased weight gain when used to reduce post weaning scouring (Poulsen, 1989; Kavanagh, 1992)
  - Growth-promoting action of zinc is found to be more efficacious when fed with copper (Hill et al., 1996)
  - Nutritional studies to determine the growth promoting effects of copper (Hahn and Baker, 1996; Carlson et al., 1995)
Examples of copper and zinc doses in the United States

- **Copper**
  - Weaned pigs @ 125 ppm
    - 5,200 tons of copper per year (swine production)
  - Feeder Cattle @ 100 ppm

- **Zinc**
  - Recently weaned pigs @ 2,500 to 3,000 ppm
  - Grower pigs @ 1,500 ppm till the nursing phase
    - 21,000 tons of zinc per year (swine production)
  - Feeder Cattle @ 22 to 32 ppm

NRC Swine, 10th Ed. 1998
NRC Cattle, 7th Ed. 2000
Aarestrup, 2006
Hasman et al., 2006
Pandora’s Box

• In Greek mythology, Pandora was given a box which she was not to open under any circumstance.

• Unable to contain her curiosity she opened it, and ‘all the evil in the world’ escaped
  – All that remained was ‘hope’

Unintended consequences of many technological fixes…

Elephant in the room?  
Monkey in the closet?

*Transferable resistance and co-resistance to antibiotics  
Toxicity concerns, especially differences among species  
Environmental consequences of heavy metals
## Heavy metal and its co-selection

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Antibiotics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>As, Cu, Zn, Mn, Co, Ag</td>
<td>Cip, Tet, Chlor, β-lactams</td>
<td>Silver, 1996; Ruiz, 2003</td>
</tr>
<tr>
<td>As, Hg</td>
<td>β-lactams, Chlor</td>
<td>Wright, 2005</td>
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<tr>
<td>Cu, Co, Zn, Cd, Ni, As</td>
<td>Tet, Chlor, β-lactams</td>
<td>Levy, 2002; Nies, 2003</td>
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<tr>
<td>Hg, Zn, Cu</td>
<td>Cip, β-lactams, Trim, Rif</td>
<td>Barkay et al., 2003</td>
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<tr>
<td>Zn, Cd, Cu</td>
<td>Coumermycin A</td>
<td>Del Castillo et al., 1991</td>
</tr>
<tr>
<td>Cu</td>
<td>Ery, Van</td>
<td>Hasman &amp; Aarestrup, 2002</td>
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<tr>
<td>Zn</td>
<td>Methicillin</td>
<td>Cavaco et al., 2010</td>
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<tr>
<td>Zn, Cd</td>
<td>Methicillin</td>
<td>Cavaco et al., 2011</td>
</tr>
<tr>
<td>Cu</td>
<td>Ery, Tet</td>
<td>Amachawadi et al., 2011</td>
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</tbody>
</table>
Heavy metals and co-selection for antibiotic resistance

- Zinc usage in piglets leading to the emergence or propagation of MRSA (Aarestrup et al., 2010)
  - czrC, a gene which confers resistance to both cadmium and zinc in MRSA (Cavaco et al., 2010)
  - Zinc resistance is strongly associated with methicillin resistance in *Staphylococcus aureus* (Cavaco et al., 2011)
Heavy metals and co-selection for antibiotic resistance

- **Copper** usage in piglets and co-selection for vancomycin resistant enterococci (Hasman and Aarestrup, 2002; Hasman et al., 2006)
  - *tcrB*, a transferable gene conferring resistance to copper is present in some European VRE strains
  - *pco*, a transferable gene conferring resistance to copper is present among Gram negatives, many of which already have high levels of innate resistance to the metal
Copper Homeostasis in *Enterococcus hirae*

Adapted from Solioz and Stoyanov, 2003
Copper resistance in *Enterococcus*

- Conferred by a transferable copper resistance gene, called *tcrB*
- First reported in *E. faecium* and *E. faecalis* in Denmark
  - Carried on a plasmid
  - Plasmid also carried genes that encode resistance to macrolides [*erm(B)*] and glycopeptides (*vanA*)

Hasman & Aarestrup, 2002; Hasman et al., 2006
Development of resistance to *tcrB*, *erm*(B), and *vanA* in Enterococci

Hasman and Aarestrup, 2005
Copper homeostasis in *E. coli*
Copper resistance in *E. coli*

- Copper resistance is inducible
- First identified on plasmid pRJ1004 isolated from *E. coli* obtained from piglets supplemented with copper sulfate (Tetaz and Luke, 1983)
- Confers resistance due to plasmid-borne copper (*pco*) resistance genes
- *E. coli* strains with the *pco* determinants can tolerate up to 5-fold higher copper concentration than wild type strains
Model for copper resistance in *E. coli*

Om = outer membrane
Im = inner membrane
Blue dots = Cu\(^{II}\) (cupric oxide)
Red dots = Cu\(^{I}\) (cuprous oxide)

Zimmermann et al., 2012
*Salmonella* and *E. coli* are highly resistant to copper

Table 2
Susceptibility of 569 bacterial isolates from livestock in Denmark to copper sulphate

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of isolates</th>
<th>MIC of copper sulphate (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td><em>Salmonella</em></td>
<td>156</td>
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<tr>
<td><em>E. coli</em></td>
<td>202</td>
<td></td>
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<tr>
<td><em>S. aureus</em></td>
<td>43</td>
<td>22</td>
</tr>
<tr>
<td><em>S. hyicus</em></td>
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<td></td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>52</td>
<td>19</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>78</td>
<td></td>
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</table>

Aarestrup and Hasman, 2004
Prevalence of \textit{tcrB} in the US

- Supplementation of elevated level of copper selected for \textit{tcrB}-mediated, copper resistant enterococci in swine and cattle
- \textit{tcrB}-positive enterococci were positive for \textit{erm}(B) and \textit{tet}(M), but not \textit{vanA}:
  - Conjugation studies showed the transferability of \textit{tcrB} gene
  - Southern blot hybridization confirms the presence of the \textit{tcrB} and \textit{erm}(B) genes on a plasmid

Amachawadi et al., 2010 & 2011
Swine study: experimental design

- 240 weaned piglets
- Randomly assigned to 6 treatments (incomplete factorial design)
  - Control: no copper + no antibiotic
  - Copper (Cu): copper at 125 ppm
  - Tylosin (Tyl): 10 mg/kg BW
  - Chlortetracycline (CTC): 11 mg/kg BW
  - Copper + tylosin (Cu + Tyl)
  - Copper + chlortetracycline (Cu + CTC)
- 5 pigs/pen and 8 pens/treatment
- Fecal samples were collected weekly for 6 weeks
Sampling schedule

<table>
<thead>
<tr>
<th>Day -14 (Feb 8)</th>
<th>Day -7 (Feb 15)</th>
<th>Day 0 (Feb 22)</th>
<th>Day 7 (Mar 1)</th>
<th>Day 14 (Mar 8)</th>
<th>Day 21 (Mar 15)</th>
<th>Day 28 (Mar 22)</th>
<th>Day 35 (Mar 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>Treatment diet</td>
<td>Control diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acclimatization Phase</td>
<td>Treatment Phase</td>
<td>Washout Phase</td>
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<td></td>
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</table>
Cattle Study (a contrast)

• To Investigate:
  – Effects of copper supplementation on the prevalence of \( tcrB \) gene
  – Co-selection of \( tcrB \) gene with macrolide and tetracycline resistance
Conclusions

• Any ‘alternative’ to antibiotics whose effects are mediated through changes to microbial flora have the potential to select for ‘resistance’
• Transferable resistance via plasmids can result in potential for co-selection of undesirable factors (e.g., antibiotic resistance, virulence & adhesion-forming factors, bio-film formation)
• A comprehensive, proactive, and holistic approach to product development and risk assessment is needed