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…

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

Source: tumblr.com

Source: Wikipedia

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)

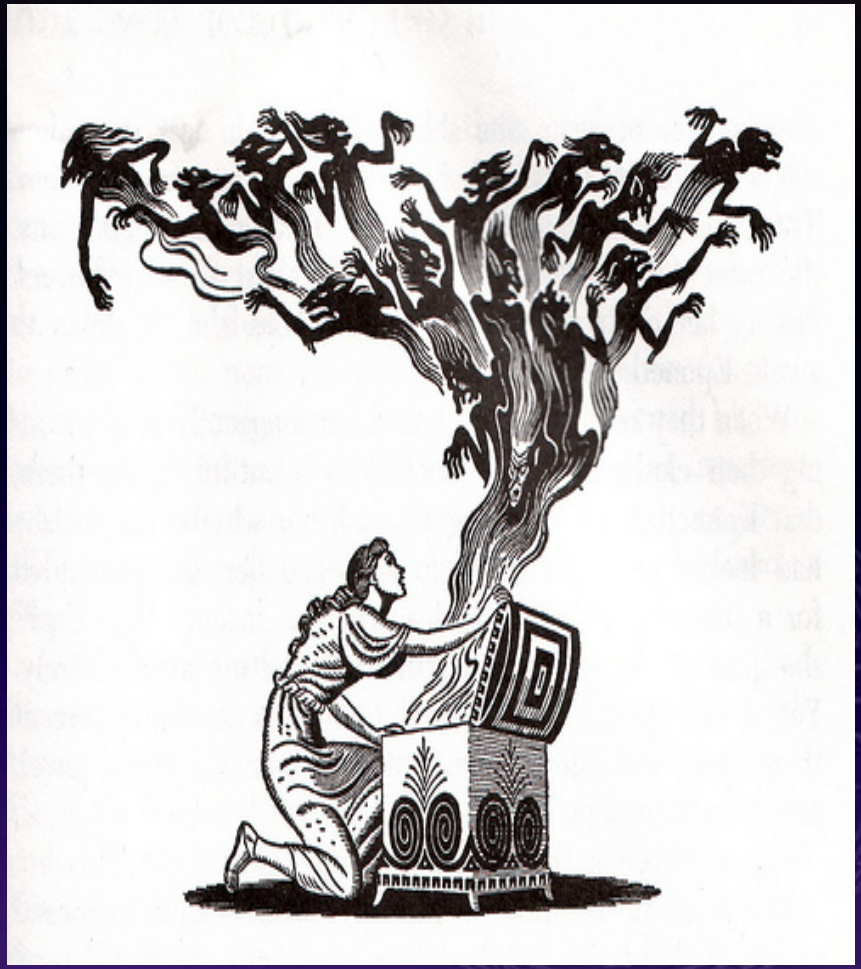
Examples of copper and zinc doses in the United States

- 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

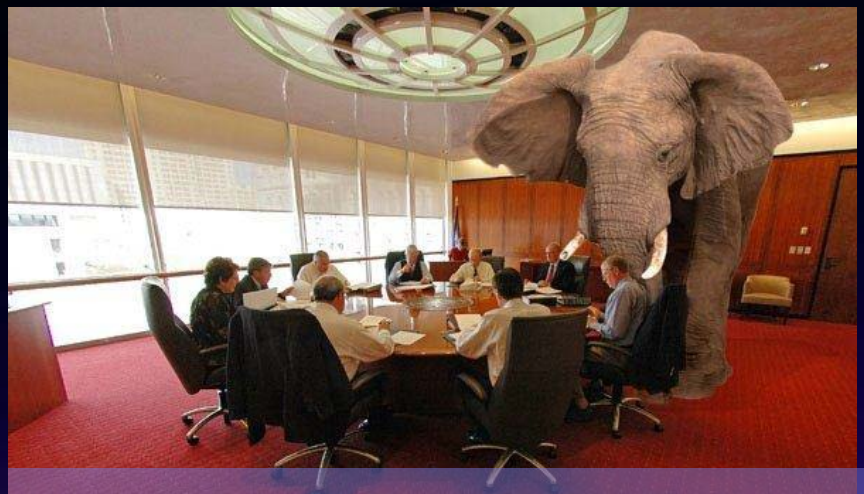
- In Greek mythology, Pandora was given a box which she was not to open under any circumstance.
- Unable to contain her curiosity she opened it, and 'all the evil in the world' escaped
  - All that remained was 'hope'



Source: Wikipedia

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

Metal ions	Antibiotics	References
As, Cu, Zn, Mn, Co, Ag	Cip, Tet, Chlor, β-lactams	Silver, 1996; Ruiz, 2003
As, Hg	β-lactams, Chlor	Wright, 2005
Cu, Co, Zn, Cd, Ni, As	Tet, Chlor, β-lactams	Levy, 2002; Nies, 2003
Hg, Zn, Cu	Cip, β-lactams, Trim, Rif	Barkay et al., 2003
Zn, Cd, Cu	Coumermycin A	Del Castillo et al., 1991
Cu	Ery, Van	Hasman & Aarestrup,2002
Zn	Methicillin	Cavaco et al., 2010
Zn, Cd	Methicillin	Cavaco et al., 2011
Cu	Ery, Tet	Amachawadi et al., 2011

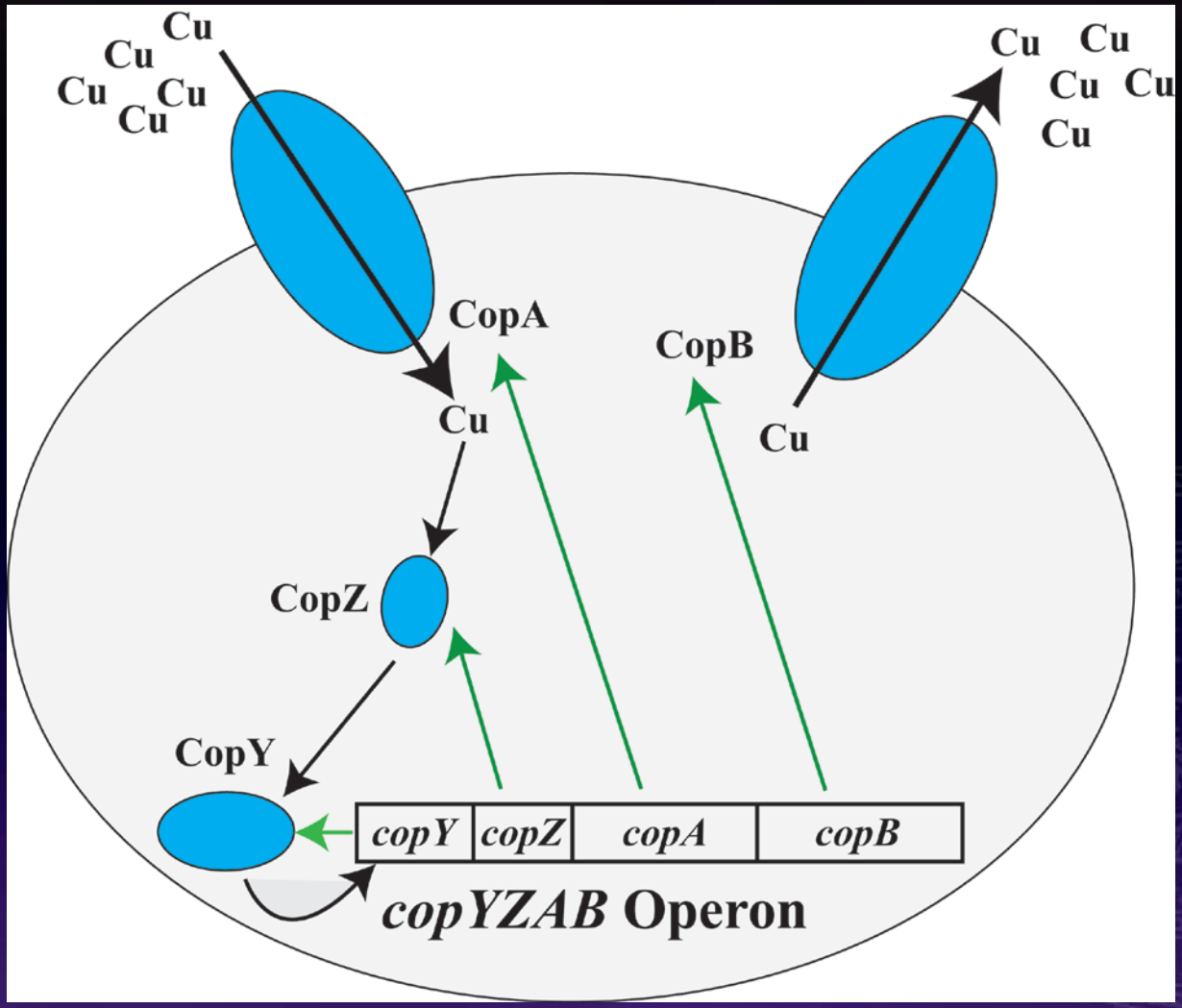
Heavy metals and co-selection for antibiotic resistance

- 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*



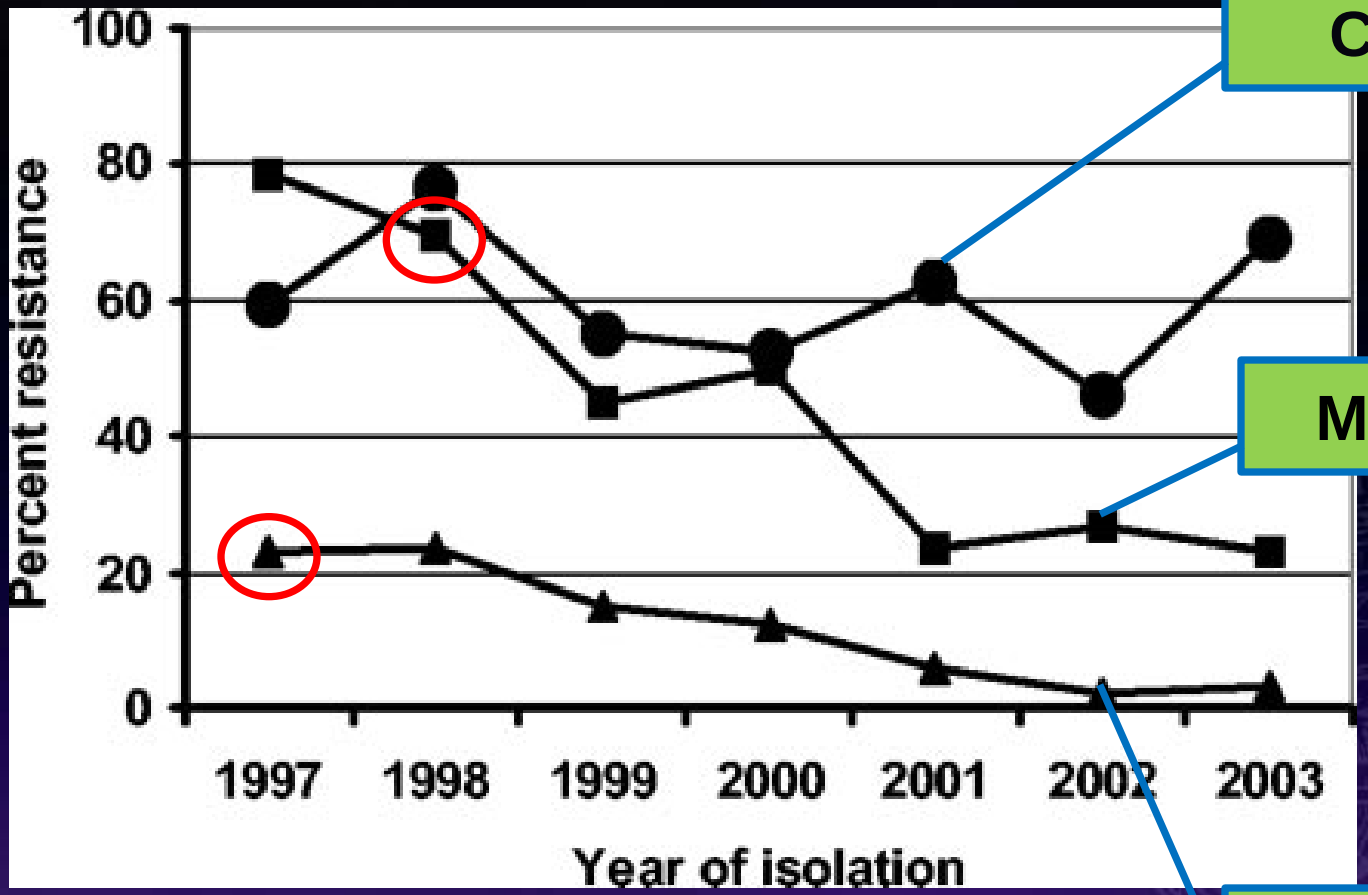
Adapted from Solioz and Stoyanov, 2003

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



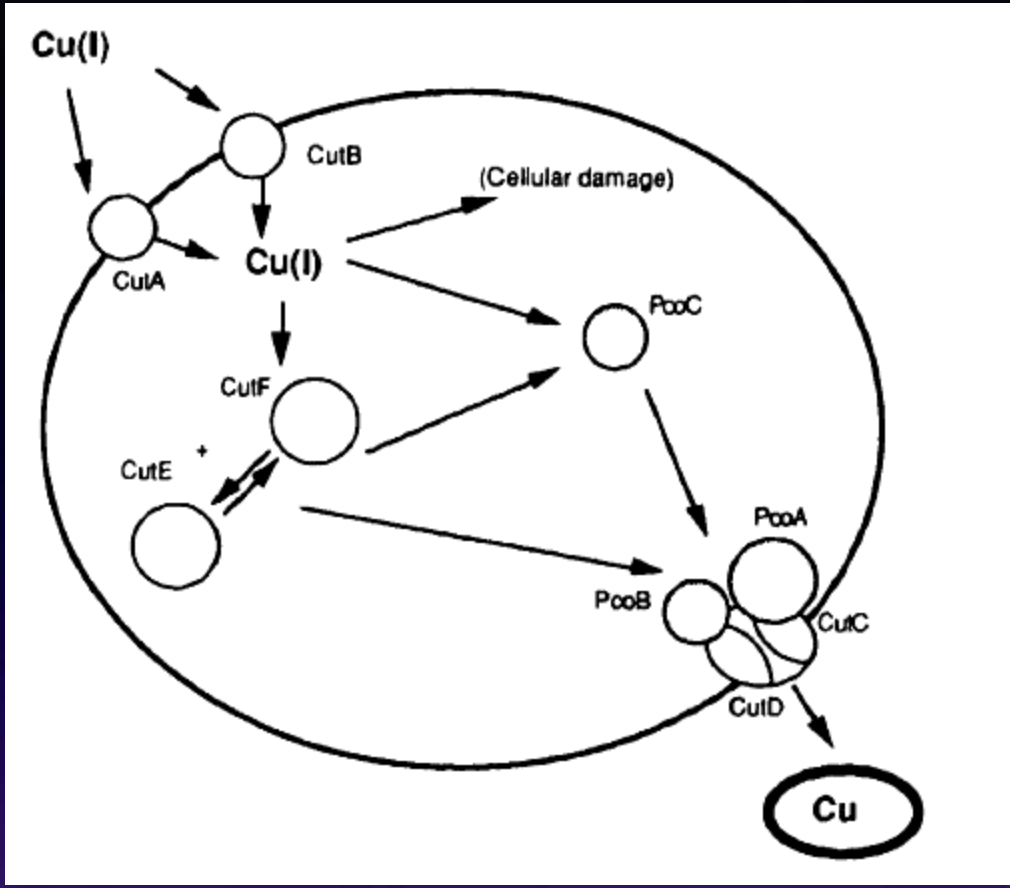
Copper

Macrolide

Glycopeptide

Hasman and Aarestrup, 2005

Copper homeostasis in *E. coli*

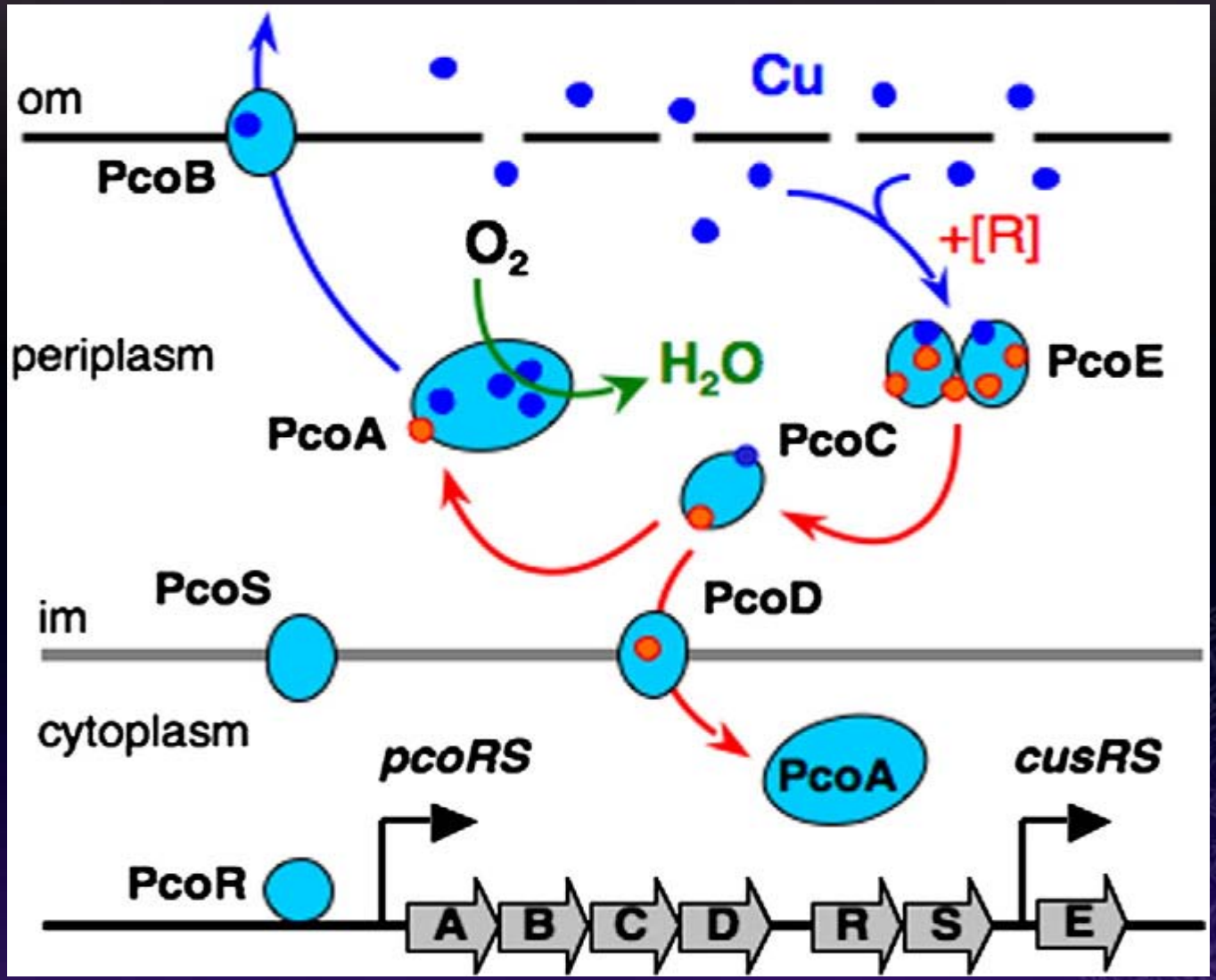


Brown et al., 1992

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 *p*lasmid-borne *co*pper (*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

Bacterial species	Number of isolates	MIC of copper sulphate (mM)								
		1	2	4	8	12	16	20	24	28
Salmonella	156							26	84	46
E. coli	202				1	5	26	169	1	
S. aureus	43		22	3	8	10				
S. hyicus	38				24	14				
E. faecalis	52		19	2		3	25	1	2	
E. faecium	78			5	8		20	3	16	

Aarestrup and Hasman, 2004

Prevalence of *tcrB* in the US

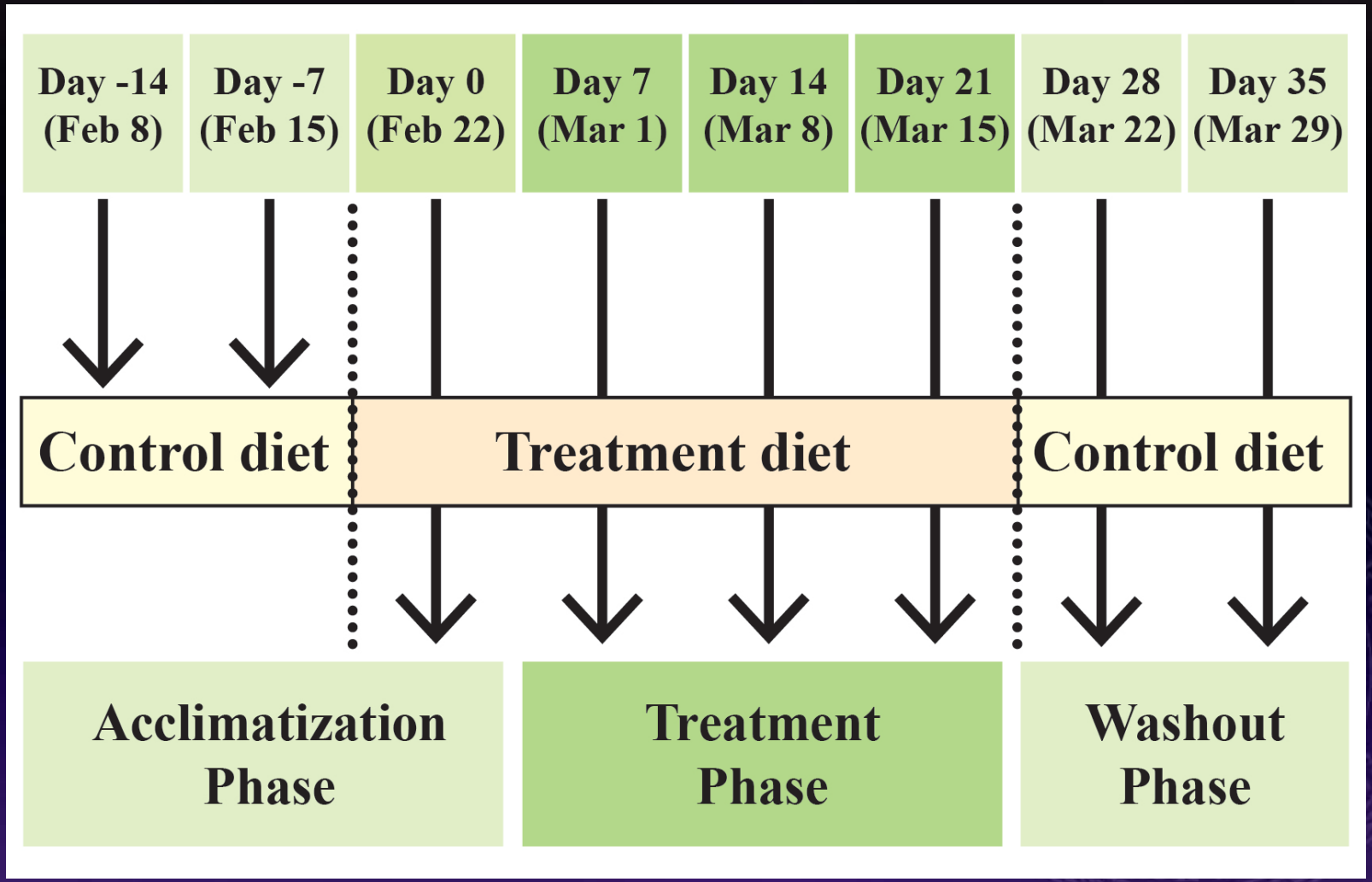
- Supplementation of elevated level of copper selected for *tcrB*-mediated, copper resistant enterococci in swine and cattle
- *tcrB*-positive enterococci were positive for *erm*(B) and *tet*(M), but not *vanA*
  - Conjugation studies showed the transferability of *tcrB* gene
  - Southern blot hybridization confirms the presence of the *tcrB* and *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

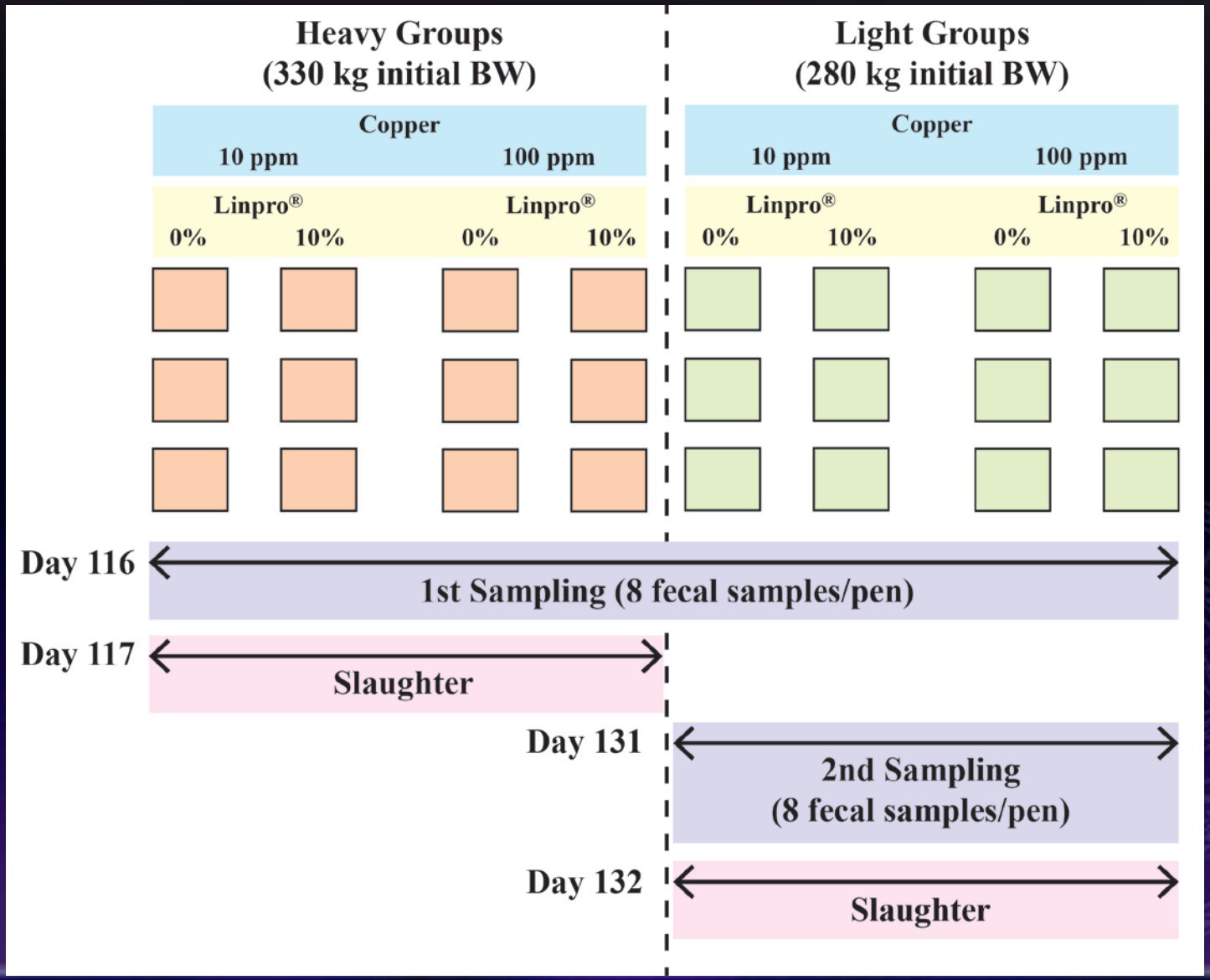
Sampling schedule



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

Experimental Design



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