Antibiotic Resistance Genes and their Association in Dairy Cattle

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Overview

• Antibiotic resistance genes (ARGs)
  ▫ What are they?
  ▫ Linked?
  ▫ Multiple resistance?

• Reservoirs in the dairy industry
  ▫ Ionophores?
  ▫ Calves?
  ▫ Cows?

• Reducing fecal ARGs
Why Are ARGs Important?

- World Health Organization (WHO), called antibiotic resistance a critical human health challenge and expressed the need for “a global strategy to contain resistance” (WHO Annual Report on Infectious Disease: Overcoming Antimicrobial Resistance)

- 2 million Americans infected, 14,000 die

- 75-95% of antibiotics can be excreted in an unaltered state (Chee-Sanford et al. 2009, Elmund et al. 1971, Feinman et al. 1978)

Why Are ARGs Important?

- Environmental Contaminant
- Animal Treatment Failure
- Human Treatment Failure
- Public Perception of the Dairy Industry

http://emmottontechnology.com
http://unmarkedfoods.com
What are ARGs? Transference?

- Segments of DNA
  - mobile genetic elements
    - Plasmids
    - Transposons
    - Integrons
- Transformation
  - Extra-cellular DNA
- Conjugation
  - Horizontal gene transfer
- Transduction
  - Bacteriophage

How are they transferred?

- Transformation
  - Extra-cellular DNA
How are they transferred?

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  - Extra-cellular DNA

How are they transferred?

- Conjugation
  - Horizontal gene transfer
How are they transferred?

- Transduction
  - Bacteriophage

  1. Phage injects its DNA
  2. Phage enzymes degrade host DNA
  3. Cell synthesizes new phages that incorporate phage DNA and, mistakenly, some host DNA
  4. Transducing phage injects donor DNA
  5. Donor DNA is incorporated into recipient’s chromosome by recombination

How do ARGs spread so readily?

- Can ARGs be linked?
- Can bacteria carry multiple resistance?
Linked genes? Multiple resistance?

- Medicated milk replacer selected for resistance genes not used on-site (Berge et al. 2006)
  - Tetracycline hydrochloride, Neomycin sulfate

- Selected for:
  - Aminoglycosides (Neomycin)
  - Chloramphenicols (Chlor 500)
  - Sulfonamides (Albon)

- E. coli Isolates
  - 39% susceptible
  - 6% resistant to one antimicrobial
  - 55% multiply resistant

Linked genes? Multiple resistance?

- Beef feedlot cattle fed tetracycline + sulfamethazine

Alexander et al. 2008
Multiple Resistance From Mastitis Causing Bacteria

- 110 cows
- 135 *E. coli* isolates
- Panel of different antimicrobials

Srinivasan et al. 2007

![Pie chart showing the distribution of genes in E. coli isolates](image-url)

Summary

- ARGs can be linked together
- High prevalence of multiple drug resistance
- Cattle are a reservoir for ARGs

http://www.futurity.org
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Where are the reservoirs?
Ionophores

• Evidence of ionophore resistance in ruminants
  ▫ 60% monensin (Dawson et al. 1983)

• Ionophore resistance is not readily spread between bacteria (Aarestrup et al. 1998, Aarestrup et al. 2000, Butaye et al. 2001)

• To date no ARG associated with ionophores have been elucidated nor do they have any effect on resistance in pathogens or their relative abundance

Where are the reservoirs?
Calves: An Important Reservoir

- Control (No antibiotics)
  - Treatment 1 (Subtherapeutic, 10mg/calf/day)
    - Neomycin sulfate + oxytetracycline hydrochloride
  - Treatment 2 (Therapeutic, 1000mg/calf/day)
    - Neomycin sulfate + oxytetracycline hydrochloride
Calves: An Important Reservoir

- Conventional farms feeding oxytetracycline
- 8 farms
- 126 calves
Calves: Intervention

Summary

- Calves fed medicated milk replacer are a significant reservoir for antibiotic resistance genes

- Antibiotic resistance genes tend to decrease throughout life

- When treatment removed antibiotic susceptibility of bacteria decrease

Kaneene et al. 2008

* - Adjusted odds ratio significant at \( p \leq 0.05 \)
Where are the reservoirs?

Lactating Dairy Cows

- 213 lactating cows
- 23 farms
- ~10% sampled from each herd
- Multiple resistance ▫ 40.48%
Lactating Dairy Cows

Sewant et al. 2007

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How Can Dairy Farmers Deal With These Issues?
Reducing Fecal ARGs

- Improve management practices in calves
  - Avoid using medicated milk replacers or waste milk containing antibiotics

- Manure management (Lactating herd)
  - Lagoons are a reservoir for ARG
  - Potential for degradation?

Reducing ARGs in Lagoon

- Aerobic & Anaerobic
  - Ab Spiked
  - Killed
  - Ab Spiked and Killed
  - Background

- Antibiotics
  - Oxytetracycline
  - Sulfamethoxazole
  - Tylosin
  - Monensin

Pei et al. 2007
Reducing Lagoon ARGs

- Antibiotic degradation occurred in all 4 antibiotics tested
- Degradation occurred
  - Best at 20°C (68°F)
- Oxytetracycline degraded completely
  - Aerobic and Anaerobic
- Time is significant when degrading antibiotics and ARGs

Pei et al. 2007

Reducing ARG by Composting

- High Intensity
  - Amended with dried leaves and alfalfa
  - Watered as necessary
  - Turned weekly
- Low Intensity
  - No amendments, water, or turning
Degradation Summary

- Antibiotics can be degraded by various methods
- ARG, time is the most important factor
Take Home Message

• ARGs are an important public health concern

• Dairy cows and calves are important reservoirs for antibiotic resistance

• Antibiotic resistance genes are organic and have the potential to be degraded

Questions?