Bacterial Antibiotic Resistance, Food Animal Production, and Human Health: No Simple Answer at The Interface of Three Complex Systems

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Infectious Disease and Antibiotics

- Infectious diseases remain the second-leading cause of death worldwide and third leading cause of death in the US.
- WHO has estimated that premature deaths would be 40% higher if antibiotics did not exist.
- Increasing prevalence of several antibiotic resistant diseases identified as high priority by Thomas R. Frieden, CDC Director, in congressional testimony (4/28/10).
  - Methicillin-resistant Staphylococcus aureus (MRSA)
  - MDR Mycobacterium tuberculosis
  - MDR Gram negatives (E. coli, Klebsiella, Acinetobacter)
  - MDR Neisseria gonorrhoeae
  - Cephalosporin-resistant Salmonella
  - Fluoroquinolone-resistant Campylobacter
- Many declarations of “critical threat to public health.”
- Fears of “return to pre-antibiotic era.”

Overused Introductory Slide at Microbiology Conferences

The History of Medicine

- 2000 B.C. – Here, eat this root.
- 1000 A.D. – That root is heathen. Here, say this prayer.
- 1850 A.D. – That prayer is superstition. Here, drink this potion.
- 1920 A.D. – That potion is snake oil. Here, swallow this pill.
- 1945 A.D. – That pill is ineffective. Here, take this penicillin.
- 1955 A.D. –Oops...bugs mutated. Here, take this tetracycline.
- 1956 - present – 39 more “oops”...Here, take this more powerful antibiotic.
- 2020 A.D.? – The bugs have won! Here, eat this root.

No Novel Antibiotics on Horizon

Timeline of introduction of antibiotic classes to clinical use

- Discovery programs have largely yielded compounds in the same class or that target the same function as known antibiotics.
- Discovery, development, and regulatory costs are extremely high with extremely high failure rates.
- Drugs with highest ROI treat chronic diseases (diabetes, etc.).

No Novel Classes of Antibiotics Forthcoming

- Thus, the challenge of antibiotic resistant infections will be met by:
  1. Preserving the effectiveness of existing antibiotics.
  3. Increasing scientific understanding of the processes contributing to the prevalence of antibiotic resistant infections.

Bacterial Antibiotic Resistance

- Bacterial antibiotic resistance is an ancient, natural, and dynamic process that pre-dates the human use of antibiotics.

So Is the ESRDA for 2009

Antibiotic resistance is ancient

Antibiotic Resistance is Diverse

- Greater than 900 β-lactamase enzymes alone.
- Bacteria outnumber us by a factor of ~10^22 and have a 3.5 billion year head start.

Antibiotic Resistance Mechanisms (Simplified)

- Antibiotic resistance occurs by four general processes:
  1. Inactivation of the antibiotic (β-lactamase).
  2. Removal of the antibiotic from cell (Efflux pumps).
  3. Alteration of of the antibiotic target (VanA vancomycin resistance).
  4. Increased production of the antibiotic target (VISA vancomycin resistance).

How Is Resistance Conferred?

- Mutation of endogenous genes (or “spontaneous mutation”),
  - Single point mutation in gyrA confers nalidixic acid resistance.
  - Antibiotic resistance in *M. tuberculosis* (TB) occurs exclusively by mutation of endogenous genes.
- Acquisition of exogenous genes encoding resistance, known as Horizontal Gene Transfer (HGT).

(Number of β-lactamase enzymes identified during the age of antibiotics)
**Horizontal Gene Transfer**

- HGT occurs by three general methods.
  - Conjugation (Plasmids, Transposons, Integrons)
  - Transduction (Bacteriophage)
  - Transformation (Free extracellular DNA)


**Antibiotic Use in Animal Agriculture**

- Concerns that antibiotic use in animal agriculture adversely impacts human health date at least to the 1969 release of the Swann Report.
- Concerns related to the “selection” of resistant bacteria that occurs when antibiotics are used.

**Antibiotic “Selection”**

- When bacteria are exposed to an antibiotic the susceptible population dies.
- Resistant bacteria may multiply to fill the space vacated by susceptible bacteria.

- This model is oversimplified.

**Antibiotic “Selection”**

- In the environment, the niche does not exist in a vacuum; other susceptible bacteria arrive following cessation of treatment.

**Routes By Which Ag. Antibiotic Use Impacts Human Health**

- “Direct” impact occurs when antibiotic use selects for an antibiotic resistant zoonotic pathogen.
Routes By Which Ag. Antibiotic Use Impacts Human Health

- “Direct” impact occurs when antibiotic use selects for an antibiotic resistant zoonotic pathogen.

- “Indirect” impact occurs when antibiotic use selects for an antibiotic resistant commensal.

Several quantitative assessments have demonstrated that the risks to human health posed by antibiotic use in animal production are low.
Avoparcin and Vancomycin are glycopeptide antibiotics with similar structures and methods of action. Avoparcin was used for growth promotion in Europe but was never used in the US. US use of vancomycin in humans was higher than in Europe. Levels of Vancomycin-Resistant Enterococci (VRE) in EU food animals, meat, and human commensal flora were higher than in US. Concluded that avoparcin use for growth promotion caused the higher levels of VRE. To lower levels Denmark banned its use in 1995 and the EU banned its use in 1997. Following the bans levels of VRE dropped in food animals, meat, and human commensal flora.

The “Precautionary Principle”

Additional bans were enacted in Denmark and the EU not to reduce levels of specific antibiotic resistant bacterial groups but based on the “Precautionary Principle.”

The Precautionary Principle states “where there are threats of serious or irreversible damage lack of scientific certainty should not postpone cost-effective measures to reduce risks to humans.”

Bans Have Not Had Desired Impact on Human Health

Denmark’s comprehensive antibiotic resistance monitoring program (DANMAP) has not observed decreases in antibiotic resistant infections in humans.

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Ag. Therapeutic Uses of Antibiotics in Denmark Increased

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Bans Have Not Had Desired Impact on Human Health

Denmark’s comprehensive antibiotic resistance monitoring program (DANMAP) has not observed decreases in antibiotic resistant infections in humans.
Pressure to Adopt EU-like Regulations Continues

- Advocacy groups continue campaigns.
- In 2012 a federal judge ordered the FDA to initiate withdrawal proceedings for growth-promoting uses of antibiotics including penicillin and tetracycline.
- In 2012 the FDA issued guidelines for the judicious use of medically important antibiotics.

GAO Report 11-801: Antibiotic Resistance

- GAO found that the current National Antimicrobial Resistance Monitoring System (NARMS) is inadequate since samples are not representative.
- NARMS is an interagency program lead by FDA, involving CDC and USDA.
- FDA's retail meat testing from 10 or 4 states is not representative.
- USDA's samples can not be used for trend analysis since they are obtained from targeted, non-representative sampling.
  - HACCP verification testing performed by FSIS is source.
- USDA's carcass sampling does not represent production environment.

Report also found that NARMS does not collect data pertaining to antibiotic use and resistance at animal production facilities.
GAO Recommendations

• To “...enhance surveillance of antibiotic-resistant bacteria in food animals...modify NARMS sampling to make the data more representative of antibiotic resistance in food animals and retail meat throughout the US.”

• To “…identify potential approaches for collecting detailed data on antibiotic use in food animals, including the species in which antibiotics are used and the purpose for their use.”

• GAO report suggests that DANMAP program could serve as model for improving NARMS.

Moving Monitoring Upstream

DANMAP & NARMS

<table>
<thead>
<tr>
<th>Entity</th>
<th>Sampled Matrix</th>
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<tbody>
<tr>
<td>Animals</td>
<td>Healthy pigs, cattle, &amp; chickens at slaughter</td>
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<tr>
<td>Animals</td>
<td>Diagnostic lab submissions</td>
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<tr>
<td>Animals</td>
<td>Targeted swine &amp; chicken herds/flocks</td>
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<td>Animals</td>
<td>Swine at processing (carcass)</td>
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<td>Food</td>
<td>Chicken</td>
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<td>Humans</td>
<td>Clinical samples</td>
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DANMAP Antibiotic Resistant Bacteria Monitoring

- DANMAP’s resistance monitoring is statistically representative.
- DANMAP includes very detailed monitoring of all antibiotic use in animals and humans.
- FDA has funded pilot studies to investigate methods for sampling of animal production environments for antibiotic-resistant bacteria.
- FSIS issued public notice #13-13 on procedures for PHVs to sample cecal contents for antibiotic-resistant bacteria.

Research Needed to Inform Regulation

- NARMS/DANMAP will never be enough.
  - NARMS and DANMAP only report on prevalence of phenotypic resistance.
  - NARMS and DANMAP do not explore genetic basis (genes) or ecology (population structures).
- New regulation is always on the horizon.

Conclusions

- Human infections complicated by antibiotic resistance are not going away nor are concerns about the contribution of antibiotic uses in animal agriculture.
- The EU & Denmark experiences illustrate that the problem will not be solved by simple solutions such as restrictions on agricultural uses of antibiotics based on the “Precautionary Principle.”
- The factors that contribute to antibiotic resistance transmission, amplification, persistence (ecology) are complex and understudied.
- The molecular mechanisms responsible for the physical transfer of MGEs (bacteriophage infection, lysis/lysogeny, transfer of conjugal plasmids, etc.) are very well understood due to 50+ years of intense study.
- Understanding of the ecology of antibiotic resistance is very limited and based on laboratory experiments.
- Studies on the ecology of antibiotic resistance in production environments may yield significant insights applicable to human medicine.
Fecal Prevalence of ESC\textsuperscript{R} E. coli

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<thead>
<tr>
<th>Month</th>
<th>No. Other Ab Injections</th>
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- IDS+TIO fecal prevalence of ESC\textsuperscript{R} E. coli was highest shortly after injection.
- IDS+TIO fecal prevalences of ESC\textsuperscript{R} E. coli were not higher than Herd prevalences during later sampling periods.

Hide Prevalence of ESC\textsuperscript{R} E. coli

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<tr>
<th>Month</th>
<th>No. Other Ab Injections</th>
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<th>IDS+TIO</th>
<th>Other + TIO</th>
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- Hide prevalence of ESC\textsuperscript{R} E. coli did not increase while at the feedlot.

Research Needed to Inform Regulation & More

- NARMS/DANMAP will never be enough.
  - NARMS and DANMAP do not report on prevalence of phenotypic resistance.
  - NARMS and DANMAP do not explore genetic basis (genes) or ecology (population structures).
- New regulation is always on the horizon.
- Industry will have to demonstrate that antibiotic uses are judicious.
- Comprehensive, longitudinal studies on the impact of specific antibiotic uses (e.g., ionophores) on specific antibiotic resistant bacteria (e.g., macrolide resistant Enterococci) are lacking.
- This data gap is exploited by the opponents of animal agriculture.
- Research can demonstrate commitment to better understanding and combating antibiotic resistance.

Acknowledgements

Meat Safety and Quality Research Unit
- Dr. Tommy Wheeler
- Dr. Terrance Arthur
- Dr. Elane Berry
- Dr. James Bono
- Dr. Mick Bosilevac
- Dr. Dayna Brichta-Harhay
- Dr. Norasak Kalchayanand
- Dr. Andy King
- Dr. Steven Shackelford
- Dr. Rong Wang
- Dr. James Wells
Mobile Genetic Elements

- Mobile genetic elements (MGE) are segments of DNA that encode enzymes that facilitate the movement of DNA.
- MGEs include bacteriophage, transposons, plasmids, insertion sequences, and integrons.
- HGT is thought to be a dominant contributor to the spread of antibiotic resistance since highly conserved antibiotic resistance genes contained within MGEs have been isolated from distantly related bacteria.

Broad Host Range IncA/C Multidrug Resistance Plasmid

- Genes for horizontal transfer.
- Genes that confer resistance to:
  - Cephalosporins (\(\text{bla}_{\text{CMY-2}}\))
  - Chloramphenicol (\(\text{floR}\))
  - Tetracycline (\(\text{tetRA}\))
  - Streptomycin (\(\text{strAB}\))
  - Sulfonamides (\(\text{sul1} & \text{sul2}\))

- Transposition/recombination

- “Backbone” sequences of IncA/C plasmids from diverse hosts are highly conserved.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Family/Order</th>
<th>Class</th>
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<tbody>
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