Multi-Drug Resistant *Salmonella*

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Salmonella and Public Health Concerns

Leading cause of death from food-borne illness

In 2002 *Salmonella* Newport and *Salmonella* Typhimurium were 2 of the top 3 Human Isolates

- Both Associated with MDR
- 27% of Typhimurium were resistant to >5 antimicrobial drugs
- 22% of Newport were resistant to >8 antimicrobial drugs
Antimicrobial Drug Resistance

Emerged as the highest priority issue for public health officials in developed countries

Antimicrobial drug resistance increases:

- Cost of health care
- Morbidity (severity and length), mortality

Resistance is not new, but have seen rapid emergence of organisms of consequence in health care

- Resistant variants of Salmonella, Campylobacter have been primary food-borne pathogens of concern
Multi-drug resistant *Salmonella*

*Salmonella Typhimurium DT104*
- Resistance genes on the chromosome
- ACSSuT resistance pattern

*Salmonella Newport (esp. MDR-AmpC)*
- Resistance plasmid/chromosomally mediated
- ACSSuTAXKGCFcCro R-Type not uncommon
- Outbreaks assoc. with beef
- *Salmonella Newport* increasingly commonly recovered from human salmonellosis

Fluoroquinolone-resist *Salmonella*
- *Fluoroquinolones primary for salmonellosis in adults*

Ceftriaxone-resist *Salmonella*
- *Ceftriaxone primary for salmonellosis in children*
Antibiotic Resistance Mechanisms

• Alteration of the drug target
• By-pass the drug target
• Inactivation of the drug
• Reduced permeability
• Increased efflux
• Increased stress regulon response
Emergence, Dissemination, Maintenance of Antimicrobial Drug Resistance

Common-place view is that:

- Genetic mutation
- Antibiotic use

While this does happen to some degree, probably represents minority of cases
In majority of instances, resistant bacteria present at very low levels in humans, animals and the environment

- Some factor allows expansion of the resistant population
- Antimicrobial use is just one factor

In general, resistance can be described as:

- Intrinsic (natural attribute)
- Acquired mutation or acquisition of DNA.
Acquired resistance

Genetic mutation

Probably a minor contributor to overall resistance

β E.g., fluoroquinolone resistance following point mutation(s) to the gyrA

• Transfer between bacteria rare but has been demonstrated qnr and proposed to become more common

β Stepwise, gradual decrease in susceptibility
Acquired resistance

Acquisition of exogenous genetic material

- DNA exchange

Likely much more common than mutations

Can occur within species & between species

Mobile genetic elements

Most important mechanism for resistance dissemination
Plasmids

Self replicating, extra chromosomal DNA

- Semi-autonomous

Commonly carry ‘adaptation’ genes

Frequently harbor resistance genes

Bacterial DNA
Plasmids

Self replicating, extra chromosomal DNA

- Semi-autonomous

Commonly carry ‘adaptation’ genes

Frequently harbor resistance genes

Plasmid-induced conjugation.

E. coli

Salmonella
Active Transfer of DNA

Well-described for plasmids

β Transposons (another mobile genetic element) can also undergo a kind of conjugation

MDR *Salmonella* Newport frequently reported to carry resistance genes on a plasmid

β *bla*\textsubscript{CMY-2} gene found on a number of plasmids

Transferable to *E. coli*

Some resistance genes are located on bacterial chromosomes while some on plasmids
Acquisition of MDR - Integrons

Act as scavengers of foreign DNA

- Allow rapid adaptation to hostile environments
- Have genetic machinery to insert and express genes

Can be located on plasmids, in transposons, or in chromosomal material

Frequently associated with development of multidrug resistance
Integrons

Exogenous DNA

Bacterial DNA
Plasmid DNA

Integron
Integrons

Genes *integrated* into DNA

May store gene cassette for future needs

Assembled cassettes can be shared between bacteria

- Conjugation i.e., plasmids, transposons
- Transduction through bacteriophages

Bacterial DNA
Plasmid DNA
Example of Integrons

*Salmonella* Typhimurium DT104

Harbors its resistance genes on 2 main integrons

- *Salmonella* genomic island 1 (SGI1)
- Acquired through bacteriophage transduction.

Integrons incorporated into chromosomal DNA

SGI1 is also found in other MDR *Salmonella* Newport
Genetic propagation

Clonal expansion

Horizontal transfer

Expansion of genes in unrelated bacteria secondary to genetic sharing
Clonal dissemination

Selection pressure applied to diverse population of bacteria

Diagram:
- Parent
  - 1st gen
    - 2nd gen
    - [Red box]
    - [Red box]
  - 1st gen
    - 2nd gen
    - [Red box]
    - [Red box]
Mobile DNA dissemination

Selection pressure applied to diverse population of bacteria

Horizontal transfer of resistance
Mobile DNA dissemination

Selection pressure applied to diverse population of bacteria

Horizontal transfer of resistance

Parent

1st gen

1st gen

1st gen

1st gen

1st gen

Parent

Donor

Recipient
Genes can be genetically linked

If use ampicillin, then select for ampicillin, streptomycin, sulfamethoxazole, and tetracycline resistance.
Potential Complexities

Genes genetically linked to other adaptive characteristics e.g., pathogenicity, acid survival, heavy metal/disinfectant resist.

If exposed to high acid, then favors survival of those with ACSSuT R types.
Salmonella serotype Newport MDR-AmpC was commonly identified… [in] humans and food animals. These findings support the possibility of transmission of this organism to humans through the food chain (Zhao et al JCM 2003)

Antibiotic-resistant strains of salmonella in the United States evolve primarily in livestock (Fey et al., NEJM 2000)

Antimicrobial resistance in Salmonella and other foodborne bacteria is largely a consequence of the use of antimicrobial agents in food-producing animals (CDC, RFP 2003)
Continued efforts to reduce unnecessary use of antimicrobial agents in food animals... [is] critical to prevent further development of resistance to quinolones and cephalosporins (Devasia et al. MDR 2005)

Broad-sweeping statements paint clear picture but not necessarily true

♫ E.g., Salmonella Typhimurium DT104
Emergence, Disappearance of DT104

DT104 was subsequently replaced by other strain types (sometimes less resistant)

No associations were made between DT104 outbreaks and antimicrobial drug use

- No antimicrobial resistance advantage over previous bovine Typhimurium clones
- Susceptible strains have emerged similarly, e.g., Typhimurium PT10 (Khakhria, *Can J Micro*, 1983)

DT104 disseminated very effectively in Norway, despite very strict controls on on-farm antimicrobial use. (*Epi Inf* 2000)
Antimicrobial Drug Use in Cattle

Subtherapeutic doses

- Prevent Liver abscesses and Improve Feed Efficiency

Therapeutic Doses

- Arrival – Prevent Infectious Diseases
- Treatment of Infectious Diseases

THEORY – Could select for Resistant bacteria that could be transferred to Humans

Little Understanding of the Link between Human MDR Salmonella and Animal Isolates
Sub therapeutic Drugs Given to Cattle

**Monensin**
- Ionophore
- 96.5% Cattle
- Improve Feed Efficiency

**Tylosin**
- Macrolide
- 42.3% Cattle
- Prevent Liver Abscesses

**Others**
- Chlortetracycline, oxytetracycline and chl and sulfamethazine
- Virginiamycin also approved – Not commonly used
Injectable

- Tilmicosin (macrolide)
- Prevent BRD or bacterial diseases – metaphylaxis - 10.4% upon arrival

Treatment – 14.4% treated for BRD

- Tilmicosin, florfenicol, enrofloxacin, oxytetracycline and ceftiofur
On-going Research

Packing plants (hide samples)
- 2 fed beef plants
- 2 cull plants
  - Cull dairy cows
  - Cull beef cows

Feedlots (6 feedlots; fecal samples)
- Healthy harvest-ready cattle
- Cull animals (realizers)

Samples collected during each of 4 seasons
- 2 seasons completed to date
On-going Research

Outcomes:

- *Salmonella* detection and quantification
- Tested against 16 USDA:NARMS defined ABX
  - Provides us ABX concentration required to inhibit bacteria. Then categorized as susceptible or not.
- Serotyping performed by USDA:NVSL

Created a bank of *Salmonella* isolates for further characterization
## Preliminary Results

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fall 2005</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Packing plant</td>
<td>Fed Beef</td>
<td>63.3</td>
</tr>
<tr>
<td>Packing plant</td>
<td>Cull dairy cows</td>
<td>75.6</td>
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<td>Packing plant</td>
<td>Cull beef cows</td>
<td>71.1</td>
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<td><strong>Winter 2006</strong></td>
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<td>50.0</td>
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</table>
## Preliminary Results

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<tr>
<td><strong>Fall 2005</strong></td>
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<td></td>
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<tr>
<td>Feedlot</td>
<td>Healthy harvest-ready</td>
<td>63.3</td>
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<tr>
<td>Feedlot</td>
<td>Cull animals (realizers)</td>
<td>23.3</td>
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<tr>
<td><strong>Winter 2006</strong></td>
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<tr>
<td>Feedlot</td>
<td>Healthy harvest-ready</td>
<td>20.0</td>
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<tr>
<td>Feedlot</td>
<td>Cull animals (realizers)</td>
<td>4.4</td>
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<tr>
<td>Source</td>
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<td>Prevalence</td>
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<tr>
<td>-----------------</td>
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<tr>
<td></td>
<td></td>
<td><strong>Fall 2005</strong></td>
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<tr>
<td>Packing plant</td>
<td>Fed Beef</td>
<td>3.3</td>
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<tr>
<td>Packing plant</td>
<td>Cull dairy</td>
<td>2.2</td>
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<tr>
<td>Packing plant</td>
<td>Cull beef</td>
<td>3.3</td>
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<tr>
<td></td>
<td></td>
<td><strong>Winter 2006</strong></td>
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<tr>
<td>Packing plant</td>
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<td>8.1</td>
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<td>Cull dairy</td>
<td>10.0</td>
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<tr>
<td>Packing plant</td>
<td>Cull beef</td>
<td>36.6</td>
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</tbody>
</table>

**MDR Salmonella**
### Quantification Data

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>Log MPN/g or 40 cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packing plant</td>
<td>Fed Beef</td>
<td>1.52 (±0.2)</td>
</tr>
<tr>
<td>Packing plant</td>
<td>Cull dairy cows</td>
<td>0.09 (±0.1)</td>
</tr>
<tr>
<td>Packing plant</td>
<td>Cull beef cows</td>
<td>0.47 (±0.1)</td>
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<tr>
<td>Feedlot</td>
<td>Healthy cattle</td>
<td>3.77 (±0.8)</td>
</tr>
<tr>
<td>Feedlot</td>
<td>Cull cattle</td>
<td>1.52 (±1.0)</td>
</tr>
</tbody>
</table>
Quantification of Data

Hide samples

Fecal samples

Animal Type

Fed Beef  Cull Dairy  Cull Beef  Healthy Feedlot  Realizers

Log10 MPN/g or 40cm²
**Salmonella Serotypes Recovered**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Fall 2005 (94%)</th>
<th>Human 2004 (60%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Montevideo (25.5)</td>
<td>Typhimurium (19.2)</td>
</tr>
<tr>
<td>2</td>
<td>Mbandaka (19.7)</td>
<td>Enteritidis (14.1)</td>
</tr>
<tr>
<td>3</td>
<td>Cerro (15.9)</td>
<td>Newport (9.3)</td>
</tr>
<tr>
<td>4</td>
<td>Anatum (15.9)</td>
<td>Javiana (5.0)</td>
</tr>
<tr>
<td>5</td>
<td>Kentucky (5.1)</td>
<td>Heidelberg (4.9)</td>
</tr>
<tr>
<td>6</td>
<td>Muenster (4.5)</td>
<td>Montevideo (2.4)</td>
</tr>
<tr>
<td>7</td>
<td>Fresno (3.8)</td>
<td>I 4,[5],12:i:- (2.1)</td>
</tr>
<tr>
<td>8</td>
<td>Havana (3.2)</td>
<td>Muenchen (2.1)</td>
</tr>
<tr>
<td></td>
<td><strong>No Typhimurium, Newport</strong></td>
<td>From CDC</td>
</tr>
</tbody>
</table>
Salmonella widely prevalent in cattle populations of the Texas High-Plains

- Frequently recovered from healthy cattle

MDR (particularly high-level) is rare in all groups.

- 68% susceptible to all; 1.7% resist 4 or more
- Most common in cull animals
  - Preliminary identification of a population at risk...

Serotypes recovered unlike those associated with human disease
Acquisition, emergence, maintenance of antibiotic drug-resistance is very complex

It is not fully understood

We do know:

- Resistance is in bacteria of livestock is uncommon
- ABX usage selects for resistance
- May be maintained without continued use of the antimicrobial drug or may disappear

Antimicrobial drug resistance is a complex and controversial issue

- Many unknowns
- Rationalization that on-farm antimicrobial drug use is the cause of epidemic dissemination of MDR Salmonella is not supported by data
International Center for Food Industry Excellence