The effects of ceftiofur and chlortetracycline treatment on antibiotic resistant *Salmonella* populations in feedlot cattle

Naomi Ohta  
Department of Veterinary Pathobiology,  
College of Veterinary Medicine and Biomedical Sciences
Salmonellosis (*Salmonella enterica*)

- **Background** -

- *Salmonella enterica*: Family of *Enterobacteriaceae*, Gram negative bacillus, with over 2,500 serotypes
- 2nd most common foodborne pathogen
- 1.2 million people get infected with foodborne *Salmonella* in the United States
- 19,000 hospitalization - 450 deaths
- Drug-resistant non-typhoidal *Salmonella* were classified as a ‘SERIOUS THREAT’ by the CDC in 2013
Cephalosporin resistant *Salmonella*

- Salmonellosis treatments for humans – **cephalosporins**, fluoroquinolones
- Ceftriaxone is used for children and high risk adults *(Critically important antibiotic, WHO)*
- Ceftiofur crystalline-free acid (CCFA: Excede®) is in the same class as ceftriaxone
- Used for bovine respiratory disease complex (BRD) treatment and control in cattle

![NARMS 2014 Graph](image.png)
Chlortetracycline (CTC: Aureomycin®)
- Top-dressed or feed-mixed supplement
- For the control and treatment of bacterial infections, such as Pasteurella spp.
  - Bovine respiratory disease (BRD)

Salmonellosis in cattle
- Subclinical infection or carriers: intermittent shedding
- Control by vaccination
- Antibiotic resistant Salmonella may be selected by antibiotics for BRD treatments?
Effects of ceftiofur and chlortetracycline on *E. coli* populations

- **Chlortetracycline treatment in feedlot cattle reduced ceftiofur resistance** *(Platt et al., 2008)*

Phenotype
- Tetracycline resistance
- Ceftiofur resistance

- **Ceftiofur followed by chlortetracycline treatment in feedlot cattle** *(Kanwar et al., 2013, 2014, Cottell et al., 2012)*

  - Tetracycline resistance: *tet*(A), *(B)*
  - Co-resistance to ceftiofur: *bla*$_{CMY-2}$
  - Ceftiofur induced more resistant isolates
Objectives of the study

**Pen level intervention that might reduce pathogens and antibiotic resistance**

1. To explore the effects of ceftiofur and chlortetracycline on the dynamics of the *Salmonella* population in the feces of feedlot cattle

2. To compare whole-genome sequencing to other approaches to classifying isolate resistance
   a. Also, comparison of serotyping results
### Treatment regimens

- Two way factorial design -

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ceftiofur (mixing)</th>
<th>CTC</th>
<th>CTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (8 pens)</td>
<td>1 out of 11 (8 pens)</td>
<td>Yes (8 pens)</td>
<td>All CCFA &amp; CTC (4 pens)</td>
</tr>
<tr>
<td>All CCFA &amp; CTC (4 pens)</td>
<td>1 CCFA &amp; CTC (4 pens)</td>
<td>No (8 pens)</td>
<td>All CCFA (4 pens)</td>
</tr>
<tr>
<td>1 CCFA (Control) (4 pens)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cattle treatments (@Canyon, TX)**
Treatment doses and days

- Methods -

CCFA: 6.6mg/kg subcutaneously base of ear

CTC: 22mg/kg feed dressing
   5 days, 1-day interval, 3 times (consistent with Platt study)

Samples tested: days 0, 4, 8, 14, 20, and 26
Salmonella isolation from feces

- Methods -

Salmonella isolation from feces involves the following steps:

1. **500mg of Glycerol Feces @ -80°C**
2. **1040 samples**
3. **TSB broth**
4. **Tetrathionate broth + iodine**
5. **Rappaport Vassiliadis broth**
6. **Brilliant Green agar**
7. **Blood agar**
8. **O-antigen test**
- Methods -

**Antibiotics Susceptibility Test**

- CLSI or NARMS breakpoints
- If resistant to ≥3 classes of antibiotics, classified as multidrug-resistant (MDR)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Range</th>
<th>Breakpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (AMP)</td>
<td>1 - 32</td>
<td>≥ 32</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic Acid (AUG2)</td>
<td>0.5 - 32/16</td>
<td>≥ 32 / 16</td>
</tr>
<tr>
<td>Azithromycin (AZI)</td>
<td>0.12 - 16</td>
<td>≥ 32</td>
</tr>
<tr>
<td>Cefoxitin (FOX)</td>
<td>0.5 - 32</td>
<td>≥ 32</td>
</tr>
<tr>
<td>Cefotaxim (TIO)</td>
<td>0.12 - 8</td>
<td>≥ 8</td>
</tr>
<tr>
<td>Ceftriaxone (AXO)</td>
<td>0.25 - 64</td>
<td>≥ 8</td>
</tr>
<tr>
<td>Chloramphenicol (CHL)</td>
<td>2 - 32</td>
<td>≥ 32</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>0.015 - 4</td>
<td>≥ 1</td>
</tr>
<tr>
<td>Gentamicin (GaN)</td>
<td>0.25 - 16</td>
<td>≥ 16</td>
</tr>
<tr>
<td>Nalidixic Acid (NAL)</td>
<td>0.5 - 32</td>
<td>≥ 32</td>
</tr>
<tr>
<td>Streptomycin (STR)</td>
<td>2 - 64</td>
<td>≥ 64</td>
</tr>
<tr>
<td>Sulfisoxazole (SOX)</td>
<td>16 - 256</td>
<td>≥ 512</td>
</tr>
<tr>
<td>Tetracycline (TET)</td>
<td>4 - 32</td>
<td>≥ 16</td>
</tr>
<tr>
<td>Oxamethrin/Sulphamethoxazole (SXT)</td>
<td>0.12/2.4 - 4/76</td>
<td>≥ 4 / 76</td>
</tr>
</tbody>
</table>
- Methods -

Serotypes, resistant genes, plasmids determination by whole-genome sequencing

- Becoming cost-effective and fast
- **Illumina MiSeq**
- Serotyping, genotyping, and finding plasmids, virulence genes detection
- DNA extraction with QIAcube HT
- Library preparation with Nextera XT kit
- Methods -

Serotyping, Resistant genes, plasmids

• De novo assembly => Velvet on BaseSpace
• Salmonella Serotyping => web based tool SeqSero (Deng et al., 2015)
• Resistant genes, plasmids, MLST => SRST2 (Inouye et al., 2014)

• Sent 10 isolates to NVSL for traditional serotyping to confirm WGS serotyping results
Statistical analysis

- Methods -

- STATA® ver. 12.1
- Bivariable analysis: Fisher’s exact test
- Mixed logistic regression analysis: Prevalence of MDR *Salmonella* by treatment and day (full factorial)
  - Day and treatments (CCFA and CTC) as main effects with pen as random effect
Descriptive statistics

- Results -

• # of fecal samples tested: 1,040
• # of *Salmonella* positive fecal samples: 566 => all sequenced
• # of phenotypically multidrug resistant (MDR) *Salmonella*: 88
- Results -

Marginal model prevalence estimates of *Salmonella* by treatment and day

Mixed logistic regression model
- Results -

Marginal model prevalence estimate of MDR *Salmonella* by day and treatment

Mixed logistic regression model
Distribution of phenotypically antibiotic resistant isolates

Graphs by CCFA, CTC treatment, and Day of samples
- Results -

Number of *Salmonella* isolates & proportion of MDR *Salmonella*

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 9</th>
<th>Day 14</th>
<th>Day 20</th>
<th>Day 27</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>n=29</td>
<td>n=29</td>
<td>n=34</td>
<td>n=32 (2)</td>
<td>n=25 (1)</td>
<td>n=26</td>
</tr>
<tr>
<td><strong>CTC</strong></td>
<td>n=31 (1) 3.2%</td>
<td>n=13 (3) 4.4%</td>
<td>n=8 (6) 75%</td>
<td>n=5 (3) 100%</td>
<td>n=9 (4) 25.0%</td>
<td></td>
</tr>
<tr>
<td><strong>CCFA</strong></td>
<td>n=23 (2) 9.9%</td>
<td>n=34 (1) 3.4%</td>
<td>n=25 (2) 8.3%</td>
<td>n=32 (2) 8.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CTC&amp;CCFA</strong></td>
<td>n=16 (1) 6.7%</td>
<td>n=18 (1) 7.7%</td>
<td>n=8 (9) 100%</td>
<td>n=8 (6) 76%</td>
<td>n=17 (2) 17.8%</td>
<td></td>
</tr>
</tbody>
</table>

- Pansusceptible *Salmonella* - Resistant *Salmonella* - MDR *Salmonella*
Most MDR isolates were *Salmonella* ser. Reading

- Results -

<table>
<thead>
<tr>
<th></th>
<th>Anatum</th>
<th>Give</th>
<th>Kentucky</th>
<th>Mbandaka</th>
<th>Montevideo</th>
<th>Reading</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multidrug resistant No</td>
<td>4</td>
<td>108</td>
<td>76</td>
<td>215</td>
<td>76</td>
<td>0</td>
<td>479</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>86</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>108</td>
<td>77</td>
<td>215</td>
<td>76</td>
<td>86</td>
<td>566</td>
</tr>
</tbody>
</table>

All NVSL traditional serotyping (White-Kaufmann-Le Minor scheme) results matched with SeqSero results

*subset of isolates*
# Results

## Phenotype and Genotype resistance

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Total isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading</td>
<td>aadA7</td>
<td>STR</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>strA/strB</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>bla\textsubscript{CMY-2} like</td>
<td>AMP, AUG2, AXO, FOX, TI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sul1/sul2</td>
<td>O</td>
<td>*3 were –</td>
</tr>
<tr>
<td></td>
<td>tet(A)</td>
<td>SOX or -*</td>
<td>(MIC: =256)</td>
</tr>
<tr>
<td></td>
<td>floR</td>
<td>TET</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CHL</td>
<td></td>
</tr>
<tr>
<td>Reading</td>
<td>aadA7</td>
<td>STR</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>aph(3')-lia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>strA/strB</td>
<td>STR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bla\textsubscript{CMY-2} like</td>
<td>AMP, AUG2, AXO, FOX, TI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sul1/sul2</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tet(A)</td>
<td>SOX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>floR</td>
<td>TET</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CHL</td>
<td></td>
</tr>
<tr>
<td>Kentucky</td>
<td>aadA7-truncated</td>
<td>STR</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>sul1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>tet(A)</td>
<td>TET</td>
<td></td>
</tr>
</tbody>
</table>

One isolate (Give) was phenotypically resistant to 7 antibiotics, but no genes were detected.
Plasmids detected in *Salmonella* isolates

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Multidrug resistant</th>
<th>I2_Delta</th>
<th>A/C2_1</th>
<th>A/C2_1</th>
<th>I1_Alpha</th>
<th>I1_Alpha</th>
<th>P1_Alpha</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>20</td>
<td>1</td>
<td></td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>82</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>83</td>
<td>4</td>
<td>20</td>
<td>1</td>
<td></td>
<td></td>
<td>117</td>
</tr>
</tbody>
</table>

Fisher's exact = 0.000

Presence of IncA/C2 plasmid significantly associated with MDR *Salmonella*
Discussion

• Treatments with ceftiofur or chlortetracycline (or both) are significantly associated with overall decrease in *Salmonella* prevalence during treatment periods
• Prevalence of *Salmonella* recovered after 2 weeks in ceftiofur treatment group
• Addition of chlortetracycline treatment to ceftiofur significantly increased the proportion of MDR *Salmonella* starting on day 8, relative to control or single treatment groups
Discussion

• Six serotypes were found
• Serotypes determined by WGS and traditional serotyping matched
• *Salmonella* serovar Reading was the dominant MDR strain
• MDR pattern was almost identical among the isolates
• 96.5% match of genotype and phenotype results
• Resistant genes were likely located on IncA/C2 plasmid
  – (sole plasmid in these isolates)
Conclusions

- The use of ceftiofur and chlortetracycline are strongly associated with a decrease in overall *Salmonella* prevalence and an increase prevalence of MDR *Salmonella*
Future studies

- Phylogenetic analysis
- Finish quantitative analysis
- Analysis of next generation sequencing data
  - SNP, evolution of strains
  - Virulence genes
Acknowledgments

Researchers
Dr. H. Morgan Scott (Texas A&M University)
Dr. Keri Norman (Texas A&M University)
Dr. Bo Norby (Michigan State University)
Dr. Guy Loneragan (Texas Tech University)
Dr. Sara Lawhon (Texas A&M University)
Dr. Henk den Bakker (Texas Tech University)

Lab members
Dr. Javier Vinasco
Ms. Roberta Pugh
All Scott lab (ME2) members for technical, moral, and mental support.

Grant Support
USDA-NIFA-AFRI
This work was supported by the USDA National Institute of Food and Agriculture, National Integrated Food Safety Initiative grant number 2010-51110-21083. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA or NIFA.

Beef Checkoff
This work has been supported in part by a contract from the Beef Checkoff.
Questions?

http://en.citizendaily.net/