Emergence of New Antibiotic Resistance? Detecting the Presence of Extended Spectrum Beta Lactamase *Escherichia coli* in Cattle

Antibiotic resistance is becoming a worldwide epidemic. One way antibiotic resistance occurs is through cross resistance transferred from food animals to humans. The intent of this project was to determine if a new type of resistance is emerging in cattle. Extended spectrum beta-lactamase (ESBL) bacteria carry a mutation that produces an enzyme that disables beta lactam antibiotics, including cephalosporins. Samples were collected from a small ranch and a large feedlot operation to determine the prevalence of ESBL *E. coli*. All locations tested showed multiple drug resistance, including resistance to ceftriaxone and ceftiofur, which are third generation cephalosporins, in indicator of ESBL presence.

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Emergence of New Antibiotic Resistance? Detecting the Presence of Extended Spectrum Beta-Lactamase \textit{Escherichia Coli} in Cattle

Sometimes the greatest discoveries are made by accident. Sir Alexander Fleming changed the world. On September 28, 1928, he did just that, he discovered the world’s first antibiotic, penicillin, growing in, of all things, mold. His discovery revolutionized modern medicine. We have come a long way in the development of antibiotics since then, but unfortunately so have the bacteria (1).

As it turned out, antibiotics were and still are one of the most important discoveries in the history of medicine. However, the misuse of antibiotics has created what is commonly referred to as super-bugs. Most infectious, disease-causing bacteria that were once susceptible are now resistant to one or more antibiotics (13).

In the 1980s a new resistance emerged in Europe, and in the mid nineties it has begun to show up in the United States. This super-bug goes by the name ESBL or \textit{Extended Spectrum Beta-Lactamase} and is believed to be even more dangerous than the infamous MRSA. So what is an ESB (23)?

ESBLs work by producing an enzyme called beta-lactamase that can break down the most commonly prescribed antibiotics in the world, the beta-lactam
antibiotics. ESBL resistance develops through a mutation carried on a plasmid.

Plasmids are circular extra-chromosomal DNA that often carry multiple drug resistances. Plasmids are transferred when bacteria conjugate or pair up. Plasmid transfer is the most common and effective mechanism of spreading resistance from bacteria to bacteria and it is not species specific. (13)

The beta-lactam antibiotics have a four-atom carbon ring structure in common. These antibiotics include penicillins, cephalosporins, monobactams and carbapenums. At first, the antibiotics were active only against Gram-positive bacteria, but they have recently developed broad-spectrum beta-lactam antibiotics active against various Gram-negative organisms. This has increased their usefulness. However, the emergence of ESBLs is limiting that usefulness. ESBLs break the 4-atom carbon structure, disabling the antibiotic. (23)

ESBL can spread through feces and direct contact between health-workers to patients or patient-to-patient. This is why nosocomial environments such as hospitals and nursing homes are prone to outbreaks. However, there are other suspicions of its spreading. The finger is beginning to point in the direction of cross contamination from animal manure. (20)
Antibiotic use in food animals accounts for over 70% of the world's antibiotic use. Livestock are given low dose antibiotics in their food to not only prevent disease but to increase growth of the animals. Many of the antibiotics used for animals are the very same antibiotics used to treat human disease. In studies, both MRSA and ESBL have been traced back to food animals. ESBL strains of *E.coli* and *Klebsiella pneumoniae* have recently been reported in food animals in the U.S. and have reached near-epidemic proportions in the UK. It is thought that ESBLs can be spread from manure that has been used as fertilizer or that has contaminated the meat itself. (20)
**Purpose**

1. Are there antibiotic resistant strains of *E. coli* found in fecal samples collected from various cattle feed lots?

2. If antibiotic strains of *E. coli* are found, will these strains also be extended spectrum beta lactamase (ESBL) bacteria?

**Hypothesis**

1. Antibiotic resistance will be found in strains of *E. coli* from cattle fecal samples.

2. Antibiotic resistant *E. coli* strains will also have the enzyme, lactamase, making them ESBL bacteria.
Procedures

1. Bacteria samples were collected from fresh manure at three different locations: a small local ranch, Feedlot-General Pens, and Feedlot-Sick Pens. A sterile cotton swab was inserted into the manure pile then placed in sterile saline for transport to Missouri Southern State University (MSSU) microbiology BSL 2 laboratory for further testing.

2. In the lab, swabs were streaked onto Eosin-Methylene Blue agar that is selective for lactose-fermenting Gram-negative bacteria. Plates were incubated for 24 hours at 37° Celsius.

3. After confirming identity of the *Escherichia coli*, colonies were transferred to Tryptic soy broth (TSB) and were incubated for 24 hours at 37° Celsius.

4. Mueller-Hinton agar plates were streaked with bacteria-containing broth, three plates per tube.

5. The Kirby-Bauer antibiotic susceptibility test was used to determine sensitivity to tetracycline, florphenicol, enrofloxacin, sulfamethoxazole/trimethoprim, ceftriaxone and ceftiboprole.

6. Plates were incubated for 24 hours at 37° Celsius and zones of inhibition were measured for each antibiotic.
## Results

**Figure 1: Comparison of Samples Taken from Small Local Ranch**

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Tetracycline</th>
<th>Sulfamethoxazole-Trimethoprim</th>
<th>Florfenicol</th>
<th>Enrofloxacin</th>
<th>Cefiotur</th>
<th>Ceftriaxone</th>
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</table>

X indicates resistant or intermediately resistant

In Figure 1 X indicates resistance or intermediately resistant. For Tetracycline 9 out of 11 bacterial samples were not susceptible to one or more of the tested antibiotics. There was no resistance seen to sulfamethoxazole/trimethoprim. For florfenicol, enrofloxacin, ceftiofur and ceftriaxone all had 8 out of 11 samples that were resistant or intermediately resistant.
Graph 1 is showing the Percentages of cattle from the Small Ranch that were not susceptible to antibiotics. Tetracycline had 82% of the cattle that were resistant or intermediately resistant. For florfenicol, enrofloxacin, ceftriofur, and ceftriaxone all had 73% not susceptible.
Figure 2: Comparison of Samples Taken from Feedlot-General Pens

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Tetracycline</th>
<th>Sulfamethoxazole-Trimethoprim</th>
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<th>Enrofloxacin</th>
<th>Imipenem</th>
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</table>

X indicates resistant or intermediately resistant

Figure 2 shows resistances seen in bacterial samples taken from a feedlot in the general pens. The feedlot was a large operation that housed approximately 18,000 head of cattle. Tetracycline resistance was seen in 4 out of 10 samples. For florfenicol 8 out of 10 samples showed resistance. For enrofloxacin, imipenem, and ceftriaxone all had 1 out of 10 that were not susceptible.
Graph 2 is showing the percentages of the samples from the feedlot-general pens that were not susceptible to the antibiotics. Tetracycline had 40% that were not susceptible. For florfenicol 80% were resistant or intermediately resistant. Enrofloxacin and ceftriaxone had 10% that were not susceptible.
In Figure 3, samples were taken from the same feedlot as figure 2 but from the hospital or sick pens where most of the cattle were taking one or more antibiotics. For tetracycline and florfenicol all of the samples showed resistance. For enrofloxacin and ceftriaxone, 6 out of 10 were not susceptible.
Graph 3 is showing the percentages of the samples from the feedlot-sick pens that are not susceptible to antibiotics. Florfenicol and tetracycline had 100% resistance. Enrofloxacin and ceftriaxone had 60% that were not susceptible.
Conclusions

An interview with the small ranch owner revealed that prior to eight years ago feeder-calves were bought from various locations in which the medical history was unknown. Eight years ago the operation changed to a cow-calf operation in which all the cattle on the ranch’s medical history was known. The cattle in the feedlot that was tested, had only been given one antibiotic, Tetracycline and yet multi-drug resistances were found, including ceftriaxone and ceftiofur which are third generation cephalosporins. This type of resistance is an indicator of ESBLs.

The antibiotics used for veterinary purposes only (enrofloxacin, florfenicol, and ceftiofur) were expected to see resistance and they all did. The antibiotics used for both humans and veterinary purposes (tetracycline and sulfamethoxazole-trimethoprim) were also expected to show resistance and they all did as well. However, antibiotics such as ceftriaxone and imipenem were not expected to show resistance because they are used for humans only, this was not the case. The ceftriaxone, a human only antibiotic, showed alarming amounts of resistance. This is an indicator of ESBLs since cattle have never been exposed to ceftriaxone, which is a beta-lactam antibiotic.
Further Research

To confirm the presence of ESBLs, recommended protocol using a 3rd generation cephalosporin in conjunction with clavulanic acid will be used on strains that showed resistance to ceftriaxone. Difference in zone diameters of greater than 5 mm between discs with clavulanic acid compared to the cephalosporin discs alone confirms strains of bacteria as ESBLs (14). In addition, more samples from other locations on the small ranch and the feed lot sick pen will be taken to determine the extent of MDR and ESBL presence in cattle from this area.

It is suspected but currently not confirmed if there is cross resistance of ESBLs between food animals and humans. Additional research as continuation of this year’s data could include the identification of the gene present in E. coli from cattle in comparison to the gene found in strains of bacteria that affect humans. This would include polymerase chain reaction and DNA electrophoresis for amplification and gene identification.
Acknowledgements

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Works Consulted:

   http://www.pbs.org/wgbh/aso/databank/entries/bmflem.html (CC)


   http://www.defra.gov.uk/animalh/diseases/zoonoses/esbl.htm (Z)


   http://www.uptodate.com/patients/content/topic.do;jsessionid=5F1784712737B38110D4 (X)

9. Bearman, Gonzalo (March 17, 2004). The ABC's of ESBL: Extended Spectrum Beta-Lactamases (everything you wanted to know but were either too scared or to lazy to ask!). Retrieved December 2, 2008, Web site: http://www.people.vcu.edu/~gbearman/Adobe%20files/ESBLgonzalo.pdf (H)


dnum (V)
