

ECOLOGY OF ANTIMICROBIAL RESISTANCE OF ENTERIC *E. COLI* AND *SALMONELLA* IN BEEF CATTLE TREATED WITH ANTIBIOTICS

Carla L. Huston¹, Richard R. Evans², R. Hartford Bailey¹,
Timothy F. Best², and John E. Huston²

¹Department of Pathobiology and Population Medicine, College of Veterinary Medicine,
Mississippi State University, Mississippi State, MS 39762

²Prairie Research Unit, Mississippi Agricultural and Forestry Experiment Station,
Prairie, MS 39756

ABSTRACT:

The objective of this study was to characterize development and dissemination of antimicrobial-resistant organisms in beef cattle treated with antibiotics. Two groups of steers were treated with antibiotics commonly used in food animals and pastured with non-treated steers. One group received an extended-spectrum cephalosporin drug (ceftiofur hydrochloride) and the other received an oxytetracycline drug. Fecal samples from 108 animals (treated and controls) were collected one week prior to antibiotic administration, and daily for one week following antibiotic administration. Fecal samples were also collected weekly for three additional weeks to monitor changes in antimicrobial resistance over time, as well as prior to the movement of animals for feeding to determine pre-market presence of resistant organisms. Fecal samples were tested for non-specific enteric *Escherichia coli* and *Salmonella* organisms. Antimicrobial susceptibility patterns of five isolates from each sample were determined. Preliminary results from 2807 *E. coli* isolates that were tested for resistance against ceftiofur, tetracycline, and enrofloxacin are available from the first study group. *Salmonella* spp were not detected in any fecal samples. Prior to treatment, 13 to 17% of fecal samples from steers contained *E. coli* isolates resistant to tetracycline. No resistance to ceftiofur or enrofloxacin was detected in any isolates from either group of animals at baseline. Little resistance to ceftiofur occurred following treatment with ceftiofur hydrochloride. Only 4% of samples contained ceftiofur-resistant *E. coli* organisms in the 3 days following treatment. Only 1 animal, a control, had more than one resistant sample during these 3 days. No resistance to ceftiofur was detected after 3 days post treatment, or in the pre-market samples. Resistance to tetracycline changed daily and did not appear to occur in any apparent pattern. At no time during the monitoring period did any isolates exhibit resistance to enrofloxacin. Preliminary findings support our hypothesis that food-producing animals properly treated with antimicrobials may develop only transient antimicrobial resistance, and therefore pose little risk towards the development of antimicrobial resistance in the human food supply. In addition, it is possible that untreated animals can acquire transient antimicrobial-resistant organisms, which can be subsequently lost when removed from the contaminated environment. It is crucial to the future of animal agriculture that veterinarians and livestock producers understand how to treat production animals in an intensive environment, while preventing the development of antimicrobial resistant bacteria and decreasing the risk of potential food-borne pathogen transmission. Thus, the results from this study may have important animal and human health implications.

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MATERIALS AND METHODS:

The objective of this study was to characterize development and dissemination of antimicrobial-resistant organisms in beef cattle treated with antibiotics. The study was designed to examine two different groups of antibiotics and used two separate groups of steers over a one-year period. The first study group (spring 2002) received an extended-spectrum cephalosporin drug (ceftiofur hydrochloride) according to the labeled dose for treatment of respiratory conditions in bovine species. The second group (fall 2002) received a long acting oxytetracycline drug also according to the labeled dose for treatment of respiratory conditions in bovine species. Since neither class of antimicrobial is labeled for prophylactic use in food animals, extended withdraw periods for market were recommended according to the Food and Drug Administration's Animal Medicinal Drug Use Clarification Act (AMDUCA) and the Food Animal Residue Avoidance Databank (FARAD) recommendations.

The first study population consisted of 48 steers with no history of antibiotic administration or prior health problems since birth. Twenty-four steers were randomly allocated into two treatment groups. Group A received the lower dose of ceftiofur hydrochloride for a longer time (0.5 mg per lb of body weight, daily for 3 consecutive days). Group B received a higher dose of ceftiofur hydrochloride for less time (1 mg per lb of body weight, repeated in 48 hours). Both doses were given subcutaneously according to labeled directions. The remaining 24 steers were also allocated into each group and pastured with the treated group to serve as untreated controls. Groups were housed in similar 25-acre pastures and received similar management at the Prairie Research Unit in Prairie, Mississippi. No direct contact was allowed between the two treatment groups. All pastures were kept void of animals for 6 months prior to the study, and steers were moved into their subsequent pastures 2 weeks prior to the beginning of the study to minimize the potential variability from acquiring resistant organisms from the environment.

The second study population consisted of 60 steers with no history of antibiotic administration or prior health problem since birth. The animals were randomly allocated into two treatment groups and two control groups and managed as described previously for the first study population. Group C received the higher dose of oxytetracycline for less time (9 mg per pound of body weight, one time). Group D received a lower dose of oxytetracycline for a longer time (5 mg per pound of body weight, daily for three consecutive days).

Fecal samples were collected from all steers one week prior to antibiotic administration to determine a baseline antimicrobial susceptibility profile for each animal. Following antibiotic administration to the treatment groups, fecal samples were collected daily from all animals (treated and untreated) during the treatment period, and daily for one week post-treatment. Fecal

samples were then collected weekly for three additional weeks to monitor changes in antimicrobial resistance over time. Furthermore, fecal samples were collected prior to movement of animals for feeding, approximately 3 months after the administration of antibiotics, to determine pre-market presence of resistant *E. coli* organisms. Separate disposable sleeves were used to rectally collect samples and place them into sterile plastic containers. All samples were transported to the laboratory in coolers and processed within 24 hours of sample collection.

All microbiological procedures were performed at the Food Safety Laboratory of the Mississippi State University College of Veterinary Medicine. Standard microbiological procedures for the detection and isolation of *E. coli* and *Salmonella* were followed. Swabs were used to inoculate MacConkey agar plates for identification of non-specific *E. coli* organisms. Once pure cultures of *E. coli* were obtained, 5 isolates from each animal were chosen to increase the probability of detecting resistant organisms per animal sample. Four grams of remaining feces were then placed in tetrathionate broth at a 1:10 dilution and incubated for 24 hours. They were next placed in Rappaport Vassiliadis broth at a 1:100 dilution for 24 hours to facilitate recovery of any *Salmonella* organisms present. Samples were then inoculated onto xylose-lysine-tergitol-4 agar to tentatively identify any *Salmonella* organisms. The Kirby-Bauer (KB) method was used to detect resistance against ceftiofur, oxytetracycline, and enrofloxacin according to the National Committee for Clinical Laboratory Standards (NCCLS). In *E. coli*, cross-resistance to such various classes of antibiotics commonly occurs. Antimicrobial susceptibility patterns were categorized into susceptible, intermediate, or resistant according to the growth zone diameter. A sample was considered resistant to an antimicrobial if at least 1 of the 5 isolates exhibited resistance or intermediate resistance to that antimicrobial.

Results from the first study group, animals treated with ceftiofur, were entered into a spreadsheet and described according to prevalence of resistant organisms. Results from animals treated with oxytetracycline are still pending. Once completed, all data will be analyzed through a statistical software program. Temporal spatial patterns of the development of antimicrobial resistance will be documented for both the treated and control animals. Antimicrobial susceptibility patterns of treated animals will then be compared to those of animals not treated with antimicrobials. Treatment groups will be further compared by dosage regimen.

RESULTS AND DISCUSSION:

The first half of the study was completed in the spring of 2002. The following results are limited to the ceftiofur-treated animals. Results from the animals treated with oxytetracycline are still pending. Susceptibility patterns were determined for 2807 *E. coli* isolates from 48 animals treated with ceftiofur hydrochloride. Although 2880 total *E. coli* isolates were anticipated (48 animals x 12 days x 5 isolates per sample), all 5 isolates from several fecal samples on the days immediately following treatment were not obtained. This was possibly due to the effects of the antimicrobials on the normal bovine gastrointestinal flora. In addition, one steer from Group B, a control animal for the low-dose group, was removed from the study one week post-treatment due to an unrelated injury requiring further veterinary care. It was expected to be able to isolate generic *E. coli* from the feces of all study animals to use as the primary indicator of antimicrobial resistance. All cattle shed multiple clones of *E. coli* at any given time, making generic *E. coli* a good target organism from which to monitor antimicrobial resistance patterns. Although few

types of *E. coli* are known to cause illnesses in humans, any of these clones could carry the genetic material which codes for antimicrobial resistance. *Salmonella* spp were not detected in any of the fecal samples, thus no resistance data are available.

As potentially zoonotic organisms, both *E. coli* and *Salmonella* were targeted for monitoring. In 1996, the Food Safety Inspection Service developed *E. coli* and *Salmonella* performance criteria for all meat and poultry processing plants. A study by Sofos, et al in the Journal of Food Protection (vol. 62(2):140-145, 1999) showed that 50-100% of beef carcasses at seven different slaughtering plants had evidence of *Salmonella* contamination, and 75-100% of beef carcasses had *E. coli* contamination. Since *E. coli* are normally found in feces of healthy animals, and *Salmonella* is the most common zoonotic food-borne pathogen associated with beef and beef products, it is likely that these organisms will enter the human food supply at some point in time. Given the fact that only apparently healthy animals are allowed to enter the food supply, it is essential to continue to monitor healthy populations of animals for development of antimicrobial resistance.

At baseline (one week prior to treatment), 17% (4/24) of fecal samples from Group A steers contained *E. coli* isolates resistant to tetracycline. Similarly, 13% (3/24) fecal samples from Group B steers contained *E. coli* isolates resistant to tetracycline. No resistance to ceftiofur or enrofloxacin was detected in any isolates from either group of animals at baseline. These baseline findings are not unusual for enteric commensal organisms such as *E. coli* since tetracycline resistance is a common phenotype found in many organisms.

Little resistance to ceftiofur occurred following treatment with ceftiofur hydrochloride. Only 4% of samples contained resistant *E. coli* organisms in the 3 days following treatment. Two fecal samples contained ceftiofur-resistant *E. coli* organisms 1-day post treatment (1 low-dose treated and 1 control animal), 1 sample contained ceftiofur-resistant organisms 2 days post treatment (control), and 3 samples contained ceftiofur-resistant organisms 2 days post treatment (1 low-dose treated and 2 high-dose treated) controls. Only 1 animal, a control, had more than one resistant sample during these 3 days. No resistance to ceftiofur was detected after 3 days post treatment. Resistance patterns to tetracyclines changed daily and did not appear to be related to ceftiofur treatment or resistance. Although tetracycline resistance did not appear to occur in any pattern, some animals appeared to have more resistance to tetracyclines than others over the entire monitoring period. It is likely that the normal flora of animals naturally vary in their susceptibility to antimicrobials. This was demonstrated in the baseline study, where 13% to 17% of samples were resistant to tetracyclines prior to any treatments. At no time during the monitoring period did any isolates exhibit resistance to enrofloxacin. Furthermore, the only resistance demonstrated in the pre-market samples was exhibited against tetracycline (19% of samples).

Although preliminary results showed little effect of treatment with ceftiofur on development of resistance in enteric *E. coli*, it is not known whether multiple treatments at a later time or the use of multiple antibiotics at one time would influence the development of resistance. Judicious use of antimicrobials is crucial in preventing the development of antimicrobial resistance. Current concern has arisen surrounding the emergence of strains of organisms that are resistant to antimicrobials used in both cattle and human populations. Tetracyclines and their derivatives,

fluoroquinolones such as enrofloxacin, and expanded-spectrum cephalosporins are classes of antimicrobials that are being closely monitored. Fluoroquinolones have been available for human use since 1986 and are the treatment of choice for serious human gastrointestinal illnesses. Fluoroquinolones are currently approved for treatment of respiratory conditions in cattle, but strict monitoring continues from both regulatory and animal health agencies. Expanded-spectrum cephalosporins, the recommended agents for invasive *Salmonella* infections in humans, are also being closely monitored for the development of resistance. The expanded-spectrum cephalosporin ceftiofur has been approved for therapeutic veterinary use in food animals and remains the treatment of choice for many conditions due to its broad spectrum and short slaughter withdraw period requirements. Tetracyclines have been widely used for many years in both human and veterinary medicine, are generally lower in cost than many other therapeutic agents, and are readily available.

Although there is little evidence to support the idea that the use of antimicrobials in food-producing animals poses a threat to public health, many call for the immediate reduction or regulation of antimicrobials in food-producing animals. Restriction or removal of entire classes of antimicrobials could seriously restrict the availability of safe and efficacious antimicrobial treatments in food animal production. Multi-resistance to antimicrobials is also a growing concern given the relative lack of new therapeutics being developed in veterinary medicine. Therapeutic failure can result in increased morbidity, mortality and treatment costs. In addition, therapeutic failure can lead to increased treatment applications or dosages, which may result in increased resistant populations of organisms. Thus, antimicrobial resistance could also potentially affect industry concerns of quality assurance such as carcass injection site lesions and drug residues. Little is known about the transfer of antimicrobial resistant organisms between animals and humans, and there is little more than circumstantial evidence that the use of antimicrobials in food animals poses a serious public health risk. There is a desperate need to continue to monitor the development and dissemination of antimicrobial resistance within both animal and human populations.

Salmonella shedding was not detected in fecal samples from any of the steers in either spring or fall testing periods. According to the 1997 USDA National Animal Health Monitoring System (NAHMS) Beef Cow-Calf survey for Southeastern states, approximately 11% of cow-calf operations had at least one *Salmonella* positive animal on the premise at any given time. Of those farms, an average of 11% of the animals were found to be shedding *Salmonella* in the feces. Although it was expected to detect the organism in a small proportion of animals, the negative results were not unusual. *Salmonella* is a common enteric pathogen found in cattle, however, fecal shedding of the organism is often intermittent, and our sampling schedule may not have been conducive to detection of the organism. It is also possible that animal density and pasture conditions did not allow for organism viability and transmission, and that negative results reflect the true herd status.

Antimicrobial resistance patterns of enteric *E. coli* organisms were monitored from animals treated with antibiotics over time. It is well known that the use of antimicrobials can lead to natural selection pressure for resistant organisms. Exposure of bacterial populations to antimicrobial drugs inhibits bacteria that are susceptible, resulting in a greater proportion of resistant isolates in subsequent generations. However, little resistance was detected in treated

animals during this study. Furthermore, no ceftiofur-resistant organisms were detected in the pre-market fecal samples taken 4 months after the start of the trial. In addition, antimicrobial resistance patterns of enteric organisms from untreated animals sharing pasture with the treated animals were monitored. This allowed us to evaluate the impact of environmental contamination and animal-to-animal transmission of antimicrobial-resistant organisms. Transmission of resistant organisms among groups of animals has been shown to occur when animals are in close contact. It is possible that animals can transiently acquire resistant organisms such as *E. coli* and *Salmonella* from their surroundings. This could mean that animals not exposed to antimicrobial treatments could develop and harbor antimicrobial-resistant organisms. However, since no resistance was detected after 3 days post-treatment in either the treated or control animals, these preliminary findings indicate that this acquired resistance does not persist.

Results presented in this report are preliminary. Laboratory results from isolates obtained from steers treated with long-acting oxytetracycline are not complete. Further in-depth analysis of this data, as well as a combined analysis of the two treatment groups, will occur when the final results are reported from the laboratory. Furthermore, the pre-market sample results will be incorporated into the final analysis. Food-producing animals properly treated with antimicrobials may develop only a transient antimicrobial resistance, and therefore pose little risk towards the development of antimicrobial resistance in the human food supply. In addition, it is likely that untreated animals can acquire transient antimicrobial-resistant organisms, which will be subsequently lost when removed from the contaminated environment. Little is known about the actual development and transmission of antibiotic resistant organisms within or among groups of animals. In addition, little is known about the persistence of resistant organisms or their potential impacts on the food production industry. Understanding the ecology of antimicrobial resistance in food animal populations will help the veterinary practitioner and producer make well informed decisions regarding the health of food animals, potentially leading to effective and practical intervention programs based upon scientific evidence. It is crucial to the future of animal agriculture that veterinarians and livestock producers understand how to treat production animals in an intensive environment, while preventing development of antimicrobial resistant bacteria and decreasing the risk of zoonotic food-borne pathogen transmission. Thus, the final results from this study may have both important animal and human health implications.

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