Association between ceftiofur use and isolation of *Escherichia coli* with reduced susceptibility to ceftriaxone from fecal samples of dairy cows

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**Objective**—To estimate the association between ceftiofur use and the isolation of *Escherichia coli* with reduced ceftriaxone susceptibility from fecal samples of dairy cows.

**Animals**—1,266 dairy cows on 18 farms in Ohio.

**Procedures**—Individual fecal samples from all cows in the study herds were tested for *Escherichia coli* with reduced ceftriaxone susceptibility. Herd antimicrobial use policy and antimicrobial treatment records were also obtained. Plasmid DNA from these isolates was tested for the presence of the AmpC β-lactamase gene (*blaCMY-2*). Minimum inhibitory concentrations to a standard panel of 16 antimicrobial drugs were determined by use of a broth microdilution system.

**Results**—Herds for which ceftiofur use was reported were more likely to have cows from which reduced-susceptibility *E coli* was isolated than herds that did not report ceftiofur use (odds ratio, 25.0). However, at the individual cow level, no association was found between recent ceftiofur treatment and isolation of reduced-susceptibility *E coli* (adjusted odds ratio, 1.01). No observed linear relationship was found between the percentage of cows from which *E coli* with reduced ceftriaxone susceptibility was isolated and the percentage of cows in the herd recently treated with ceftiofur.

**Conclusions and Clinical Relevance**—Our observation of a herd-level but not an individual cow-level association between ceftiofur use and isolation of *E coli* with reduced ceftriaxone susceptibility from fecal samples suggests that interventions to reduce the spread of antimicrobial resistance genes in agricultural animals will be most effective at the herd level. (Am J Vet Res 2006;67:1696–1700)

The emergence of bacterial pathogens that are resistant to medically important antimicrobial drugs is a current public health concern. Considerable debate exists regarding the extent to which the use of antimicrobial drugs in food animals, particularly at subtherapeutic doses, contributes to the development and dissemination of antimicrobial-resistant organisms with public health implications. Also, some debate exists regarding the appropriateness of the use of antimicrobial drugs classified as critical to human medicine, such as fluoroquinolones and expanded-spectrum cephalosporins, in food animals. When food animals receive antimicrobial drugs, resistant organisms can develop, as with any individual receiving antimicrobial treatment. However, the development of resistant bacteria in food animals is especially worrisome because these bacteria have the potential to be widely disseminated to humans through the food supply.

One antimicrobial drug that has received considerable attention is ceftiofur, a third-generation cephalosporin that is labeled for veterinary use in food and companion animals. Ceftiofur is approved for veterinary use in food animals to treat a variety of gram-negative bacterial infections, including important health conditions such as bacterial respiratory diseases that can cause production loss. Ceftiofur is quickly metabolized and has the food safety benefit of low risk for residues in meat that exceed allowable amounts. In addition, parenterally administered ceftiofur does not produce residues in milk that exceed allowable amounts. As a result, milk from dairy cows treated with this drug does not need to be discarded, as is the case with most other antimicrobials. Because of this low risk for milk residues as well as its perceived efficacy, ceftiofur is used to treat a variety of common health conditions in lactating dairy cows. Ceftiofur is similar to ceftriaxone, an expanded-spectrum cephalosporin drug used in human medicine. Both are third-generation cephalosporin drugs and are similar in molecular structure and bactericidal activity. Ceftriaxone is approved to treat gram-negative bacterial infections in humans and is the antimicrobial of choice for treatment of invasive gram-negative bacterial infections, including salmonellosis in children. Several authors have hypothesized that the widespread use of ceftiofur on farms has resulted in zoonotic foodborne transmission of ceftriaxone-resistant enteric bacteria. However, no data are currently available describing the direct impact of ceftiofur use on the development and dissemination of bacterial resistance in animals and animal populations. A better understanding of this impact is important in determining whether ceftiofur use on farms is a public health risk. Thus, we designed this study to estimate...
the association between ceftriaxone use and the isolation of *Escherichia coli* with reduced ceftriaxone susceptibility from fecal samples of dairy cow populations.

**Materials and Methods**

**Animals**—The study population consisted of a convenience sample of 18 dairy herds located throughout Ohio. These herds were identified because they were involved in other surveillance projects with the investigators, were herds belonging to the Ohio State University, or were clients of the Ohio State University College of Veterinary Medicine. Although this sample of herds is not a random representation of all dairy farms in Ohio, the management of these herds may be typical of Ohio dairy farms. Antimicrobial treatment policy for each dairy farm was obtained, including reported ceftriaxone use. In addition, detailed antimicrobial treatment records of individual cows for the 6-month period prior to sample collection were obtained for a subset of the dairy herds that maintained these records.

**Sample collection and processing**—Fecal samples were collected from all lactating and nonlactating cows present in each of the participating herds. Approximately 20 g of feces was collected rectally from each cow by use of individual plastic collection bags placed and sealed in sterile 50-mL plastic centrifuge tubes. These samples were transported to the laboratory at ambient temperature, with transport time ranging from approximately 15 minutes to 2 hours. One gram of fecal material from each of the samples was incubated overnight in 10 mL of nutrient broth containing cefoxitin (4 μg/mL) at 37°C. Each sample was then streaked onto MacConkey-4-methylumbelliferyl-β-D-glucuronide agar containing ceftriaxone (8 μg/mL) and incubated overnight at 37°C. Colonies that were lactose and glucose-positive were presumed to be *E. coli*. A single characteristic colony was selected to represent each sample with a positive result. Plasmid DNA was isolated from each of these *E. coli* isolates by use of a commercial kit and tested for the presence of the AmpC β-lactamase gene (*blaCMY-2*) by use of a PCR procedure described for previous studies. Minimum inhibitory concentrations to a standard panel of 16 antimicrobial drugs were determined by use of the semiautomated broth microdilution system following Clinical and Laboratory Standards Institute guidelines. American Type Culture Collection *E. coli* 25922, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 27853 were used as quality-control organisms.

**Data analysis**—The outcome of interest was the detection of *E. coli* with reduced ceftriaxone susceptibility in the intestinal flora of the study cows; reduced susceptibility was defined as a minimum inhibitory concentration of ≥ 8 μg/mL. Data were examined at the herd level and individual cow level. Herds were classified as positive if reduced-susceptibility *E. coli* was isolated from their fecal sample. Herds were also classified on the basis of the reported ceftriaxone use policy into those that did and did not use ceftriaxone. Cows were classified as positive if reduced-susceptibility *E. coli* was isolated from their fecal sample. Additionally, the presence of *E. coli* with reduced ceftriaxone susceptibility and treatment with ceftriaxone was examined at the herd and individual cow level. The Pearson χ² test was used to examine the herd-level association between reported use of ceftriaxone on the farm and the isolation of reduced-susceptibility *E. coli*. The Spearman rank correlation coefficient was used to assess the herd-level correlation between percentage of cows from which reduced-susceptibility *E. coli* was isolated and percentage of cows treated with ceftriaxone. Logistic regression was used to examine individual cow-level association between ceftriaxone treatment and the isolation of reduced-susceptibility *E. coli* within herds that reported cows treated with ceftriaxone. The model included the herd in which the cow was located as a random effect variable by use of the generalized estimating equations method with an exchangeable correlation structure. In addition, a multilevel logistic regression model was constructed with software to estimate the proportion of total variance in the odds of isolation of a reduced-susceptibility *E. coli* that could be attributed to herd-level and individual cow-level variability. For assessment of the proportion of variance attributable to the levels of organization, the value of χ² was assigned to the lowest level (fecal sample from a cow) for all models. Values of *P < 0.005* were considered significant.

**Results**

A total of 1,266 fecal samples were collected, with samples collected from a mean of 70 cows for each of the 18 study herds (range, 23 to 159 cows). Eleven of the 18 dairy herds reported the use of ceftriaxone. Eight of the 18 dairy herds had complete cow treatment records that were used for cow analysis. These 8 dairy herds, all of which reported ceftriaxone use, included 782 cows. Of the 782 cows, 107 (13.7%) received treatment with ceftriaxone in the 6 months prior to the fecal sample collection, whereas 675 (86.3%) did not receive ceftriaxone treatment. The mean percentage of cows treated with ceftriaxone in these 8 herds was 12.4% and ranged from 0% to 35.8% in the previous 6 months. One of these 8 herds reported a policy for the use of ceftriaxone but had not had a cow requiring ceftriaxone treatment in the past 6 months.

*Escherichia coli* with reduced ceftriaxone susceptibility was isolated from 12 of the 18 herds. These *E. coli* isolates were recovered from ≥ 1 cow in 10 of 11 herds reporting ceftriaxone use and from 2 of 7 herds reporting no ceftriaxone use. From the 1,266 individual fecal samples collected, *E. coli* with reduced ceftriaxone susceptibility was isolated from 436. Thus, the overall prevalence of reduced-susceptibility *E. coli* among the cows tested was 34.4%. The observed prevalence within individual dairy herds ranged from 0% to 97% in herds that reported ceftriaxone use and from 0% to 34% in herds that reportedly did not use ceftriaxone. The mean herd prevalence was 40% for herds that reported ceftriaxone use but only 9% for herds that reported no ceftriaxone use. Of the 436 cows from which reduced-susceptibility *E. coli* was isolated, a single isolate from each of 429 cows was examined for *blaCMY-2*. Of the 429 isolates, 353 (83%) were found to contain *blaCMY-2*. We did not test for the presence of other bacterial resistance genes that might confer resistance to expanded-spectrum cephalosporin drugs. Minimum inhibitory concentrations to a standard panel of antimicrobial drugs were determined for a subset of 81 isolates with *blaCMY-2*; isolates were selected to represent each of the dairy herds considered positive for reduced-susceptibility *E. coli*. Of these, all were resistant to amoxicillin-clavulanic acid, ampicillin, cephalexin, cefoxitin, ceftriaxone, streptomycin, sulphonamide, and tetracycline. In addition, 53% (43/81) of the isolates were resistant to ceftriaxone, whereas all had reduced ceftriaxone susceptibility (minimum inhibitory concentra-
tion, > 8 μg/mL). The percentage of isolates classified as susceptible, intermediate, or resistant to each of the drugs was determined (Table 1).

Dairy herds in which ceftiofur use was the reported policy were more likely to have cows from which we isolated reduced-susceptibility *Escherichia coli* than those that did not use ceftiofur (odds ratio, 25.0; *P* = 0.01; 95% confidence interval, 1.29 to 1121.5). However, at the individual cow level, no association was found between ceftiofur treatment and isolation of reduced-susceptibility *E coli* adjusting for the confounding effects of the herd in a logistic regression model. Also, no observed linear relationship was found between the percentage of cows from which we isolated reduced-susceptibility *E coli* and the percentage of cows treated with ceftiofur in the herd (*r* = 0.19; *P* = 0.47; Figure 1). Most (71%) of the variability in the odds of isolation of a reduced-susceptibility *E coli* was observed at the herd level.

### Discussion

It has been speculated that the use of ceftiofur in agricultural animal populations has resulted in the zoonotic foodborne transmission of ceftriaxone-resistant organisms of public health concern. However, little data currently exist that describe the impact of veterinary ceftiofur use on the development and dissemination of resistant bacteria. Available data from the National Antimicrobial Resistance Monitoring System suggest that the prevalence of ceftriaxone-resistant organisms in human and animal populations is increasing, but the importance of ceftiofur use in veterinary medicine on this increase has not been established. To establish the public health importance of agricultural ceftiofur use, data are needed regarding ceftiofur use on farms and its association with the dissemination of expanded-spectrum cephalosporin bacterial resistance genes within animal populations. Generating data to address this issue was the objective of our study. However, data will also be needed to establish the frequency that these bacterial resistance genes actually enter the food supply, the frequency with which they are acquired by consumers from the food supply, and the frequency which they result in undesirable health outcomes in humans. These data are not yet available.

We found that 61% of the dairy herds reported that ceftiofur was used to treat some health conditions. The mean percentage of cows treated with ceftiofur was 12.4% in herds in which use was reported, ranging from 0% to 36%. Our data agree with other reports that ceftiofur is frequently used in dairy herds, although its use appears to vary between herds. We observed that in some herds, more than a third of the cows received ceftiofur during a 6-month period. This may be because, unlike most antimicrobial drugs, ceftiofur has no milk-withholding period. Thus, milk from treated dairy cows does not have to be discarded for a mandatory period after treatment. Management of antimicrobial-treated cows is much simpler without a

### Table 1—Percentage of 81 *Escherichia coli* isolates containing blaCMY-2 that were classified as susceptible, intermediate, or resistant to a panel of antimicrobial drugs.

<table>
<thead>
<tr>
<th>Antimicrobial drugs</th>
<th>Interpretive breakpoints (μg/mL)</th>
<th>Isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Amikacin</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>8/4</td>
<td>32/16</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>32</td>
<td>512</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>2/38</td>
<td>4/76</td>
</tr>
</tbody>
</table>
These data suggest that use of ceftiofur on farms leads to a higher prevalence of organisms with reduced susceptibility than those that did not use ceftiofur. In our study, 1 cow in 67% of dairy herds had an isolate with reduced ceftriaxone susceptibility, compared with 1 cow in 25% of dairy herds that did not use ceftiofur. This is in agreement with other studies. 

From which we isolated E coli were approximately 25 times as likely to have cows with reduced ceftriaxone susceptibility than those that did not use ceftiofur. An E coli isolate with reduced ceftriaxone susceptibility was isolated from ≥ 1 cow in 67% of dairy herds. The observed prevalence among cows was as high as 97% in some herds. The USDA reported in 2002 that resistance to ceftriaxone and ceftiofur for Salmonella isolates from healthy slaughter cattle was 16.6% and 0.2%, respectively. Our results are not directly comparable to the National Antimicrobial Resistance Monitoring System reported prevalence because we used a selective process for detecting these isolates and a higher prevalence is to be expected. However, it is clear that these bacterial resistance genes are commonly present in the fecal flora of Ohio dairy cow populations. The relatively high prevalence in cattle suggests that the foodborne zoonotic transmission of these resistance genes could occur because cull dairy cows often enter the food supply as fresh ground-meat products. However, because we did not quantify these resistant organisms in fecal samples, we cannot speculate as to the frequency of meat contamination during processing of an animal with these bacterial resistance genes in its fecal flora. It is possible that our selective culture procedures allowed us to recover resistant organisms that were actually rare in the flora. Thus, the possibility of resistant organisms from these cows entering the food supply cannot be estimated from these data.

Dairy herds in which ceftiofur use was reported were approximately 25 times as likely to have cows from which we isolated E coli with reduced ceftriaxone susceptibility than those that did not use ceftiofur. These data suggest that use of ceftiofur on farms leads to selection for expanded-spectrum cephalosporin bacterial resistance genes, such as blaCMY-2, in the intestinal flora of food animal populations. However, at the individual cow level, no association was found between receiving ceftiofur in the previous 6 months and the isolation of E coli with reduced ceftriaxone susceptibility. Our observation of a herd-level association but not an individual cow-level association suggests that the treatment of an individual cow with ceftiofur readily leads to the widespread dissemination of expanded-spectrum cephalosporin bacterial resistance genes to the entire population of cows. This is corroborated by our observation that most of the variability (71%) in the likelihood of recovering a reduced-susceptibility E coli was observed on the herd level rather than at the individual cow level. It is likely that cows in close contact readily share intestinal flora, allowing bacterial resistance genes to be easily disseminated to the entire herd by cows treated with antimicrobial drugs. Thus, interventions intended to reduce the spread of bacterial antimicrobial resistance genes in the flora of agricultural animals might be most effectively applied at the herd level. Approximately 83% of E coli isolates with reduced ceftriaxone susceptibility isolated in our study were found to contain blaCMY-2, which is a gene commonly found on a large plasmid in gram-negative bacteria. Large plasmids carrying blaCMY-2 have been shown to be readily transmitted from commensal organisms to pathogens. Because of the mobility of this plasmid, it is possible that E coli is not the reservoir for this resistance gene in the flora and that the E coli we observed in our study may have acquired the plasmid carrying the resistance gene during the enrichment step of our selective protocol. Organisms with this genotype are typically resistant not only to cephalosporins, but also to most β-lactam antimicrobials and are often resistant to tetracyclines, aminoglycosides, sulfonamides, and chloramphenicals. They are, however, typically susceptible to amikacin, apramycin, and ciprofloxacin. Widespread dissemination of potential human pathogens with this genotype in the food supply would not be favorable to public health.

Our observation that ceftiofur use is associated with the presence of expanded-spectrum cephalosporin bacterial resistance genes in dairy herds suggests that reduced use of this drug may be a helpful intervention to minimize the dissemination of bacterial resistance. Ceftriaxone is a prescription drug, but currently no limitations exist on its extralabel use by veterinarians. Therefore, limitations on the extralabel use of ceftiofur may be a means to reduce use of this drug by increasing veterinarian control over its administration. This method has been applied to control the use of enrofloxacin in cattle while maintaining it in the drug arsenal of veterinarians. The absence of a milk withholding time can make ceftiofur the drug of choice for dairy producers. However, if producers are allowed to make this treatment decision on the basis of withholding time or other economic reasons without direct veterinary supervision, then prudent use guidelines may be inadvertently violated. Requiring greater veterinarian control of the administration of ceftiofur may be a means to ensure the long-term availability and effectiveness of this drug to veterinarians and producers while alleviating public health concerns.

Our data suggest that simply reducing the number of cows in a herd that are treated with ceftiofur will be ineffective in reducing the spread of expanded-spectrum cephalosporin resistance genes within animal populations. Our observation of a herd-level but not an individual cow-level association implies that interventions to reduce the spread of antimicrobial resistance genes may be most effective if applied at the herd level. This suggests that for reduced ceftiofur use to be effective in reducing the dissemination of expanded-spectrum cephalosporin resistance genes, the number of herds to which the drug is administered must be limited. Thus, prudent use of antimicrobial drugs in veterinary medicine must be considered together with the potential food safety benefit of reduced antimicrobial residues associated with ceftiofur use, compared with many other antimicrobial drugs approved for dairy cows.
nary medicine might include consideration of this issue before administration of an antimicrobial drug to an animal population for the first time.

Ceftiofur has been reported to be used frequently on dairy farms for the treatment of a variety of health conditions. Thus, widespread dissemination of expanded-spectrum cephalosporin resistance genes in the flora of animals destined for the US food supply as fresh meat products may occur. Our data provide no evidence, however, that resistant bacteria present in the fecal flora of food animals will contaminate retail food products, as we did not sample these. In fact, we used selective microbiologic methods and did not attempt to quantify the resistance genes in the flora of these cows. As a result, the risk of these resistance genes entering our food supply may be small. However, it may be appropriate to consider potential interventions to limit the widespread dissemination of expanded-spectrum cephalosporin bacterial resistance genes in food animal populations. Our data suggest that reduced ceftiofur use, and possibly other interventions to reduce resistance, is likely to be most effective if applied at the herd level. It may be appropriate to consider modifying veterinary prudent use guidelines for antimicrobial drugs to include limitations on the introduction of previously unused antimicrobial drugs into animal populations.

References

2. Antimicrobial resistance. Data to assess public health threat from resistant bacteria are limited. HEHS/NSIAD/RCED-99-132.