
Use of Antibiotics in a Dairy Herd and Their Effect on Resistance Determinants in Enteric and Environmental Escherichia coli

L. D. ROLLINS, D. W. POCURULL, and H. D. MERCER
Food and Drug Administration, Division of Veterinary Medical Research, Beltsville, Maryland 20705
R. P. NATZKE1 and D. S. POSTLE2
1Department of Animal Science and 2College of Veterinary Medicine, Cornell University, Ithaca, New York 14850

Abstract
The frequency of antimicrobially resistant Escherichia coli in a dairy herd and its immediate environment was studied. The herd was given doses of 1,000,000 units of penicillin and 1 g of dihydrostreptomycin during a dry-cow mastitis treatment program. Over 1 yr, the incidence of E. coli resistant to dihydrostreptomycin (25 μg/ml) was determined for fecal and environmental samples. Antimicrobial susceptibility patterns and minimum inhibitory concentrations for dihydrostreptomycin were determined on E. coli isolates from these samples. Environmental samples were collected at 2-wk intervals from holding areas, and fecal samples were collected from cows before treatment and at 2 and 6 wk post-treatment. The entire herd was sampled initially and at the end of the test period. Peaks of resistant E. coli of 10% from both cow and environmental sources had a marked pattern of similarity, indicat-

Received November 29, 1973.

JOURNAL OF DAIRY SCIENCE VOL. 57, NO. 8
ing an enteric flora-environmental flora interaction. The therapeutic use of antibiotics, including their use in large doses in a dry-cow mastitis treatment program, resulted in minimum effects on resistance and minimum inhibitory concentrations of *E. coli*.

The dairy herds represented the model situation where antibiotics are used primarily parenterally for therapeutic purposes. Results contrast sharply as compared to drug resistance in the enteric flora of animal herds that conventionally have received antibiotics continuously in feed.

---

**Introduction**

The use of certain antibiotics in animal feeds results in a high incidence of gram-negative enteric microorganisms with transferable drug resistance. Both therapeutic and subtherapeutic usages provide selective pressure whereby drug-resistant organisms emerge. Antibiotic usage patterns have been related to the incidence (5) of drug resistance in enteric microorganisms, and their persistence (2, 3, 8, 9, 10) after cessation of antibiotic administration may be associated with usage patterns. On farms where animals were exposed continuously to subtherapeutic antimicrobials in their feed, the incidence of resistant enteric organisms and the frequency of multiple resistance were greater than on farms where antimicrobials were used intermittently for therapeutic purposes (5). When oral antibiotic administration was of short duration in experimental groups of pigs (2), calves (3), and poultry (8), the resultant high incidences of resistant organisms soon decreased. Conversely, on farms where antibiotics were fed continuously to all swine for 2 to 3 yr, a high incidence of resistant organisms persisted for the 7 mo in which they were examined (10).

In dairy herds in the United States antimicrobial drugs are used primarily for therapeutic purposes and are usually administered parenterally or by intramammary or intrauterine infusion over short periods. In contrast, antimicrobial drugs are administered continuously in feed in subtherapeutic amounts for long periods for management purposes in swine, poultry, and beef cattle herds. One of the more consistent uses of antimicrobials in dairy herds is a dry-cow mastitis therapy program in which every cow is treated at the end of lactation. The recommendation for one experimental dry-cow mastitis treatment program is the intramammary infusion of 1 million units of penicillin and 1 g of dihydrostreptomycin (DSM) into each quarter. Penicillin and DSM are absorbed from the mammary gland and excreted via the urine (7). Since large quantities of antibiotics are recommended in this particular dry-cow program, there was concern about effects of excretion of large quantities of the drugs on the coliform flora of the surrounding environment. If cows were treated at frequent intervals, the excreted drug could represent a continual selective pressure for the emergence of antibiotic-resistant organisms. Such a build-up of drug-resistant bacteria could represent a problem in the treatment of mastitis caused by resistant organisms such as *Escherichia coli*.

The influence of antimicrobial usage pattern on the incidence of drug resistance in the enteric flora of the cows and their immediate environment was studied in dairy herds where 1 million units of penicillin and 1 g of DSM were administered per quarter in dry-cow mastitis treatment. Usage of antimicrobial drugs in dairy herds also represents a model situation where these drugs are usually administered parenterally or by intramammary or intrauterine infusion in therapeutic amounts for short periods. These studies were also to compare results with antimicrobials administered continuously in feed in subtherapeutic amounts for longer periods.

**Materials and Methods**

Preliminary studies were on 10 dairy farms in New York. On five farms the dry-cow mastitis treatment program was intramammary infusion of 1 million units of penicillin and 1 g of DSM per quarter and had been for the previous 3 to 4 yr (6). Five farms were not using this specific dry-cow treatment. Ten samples were collected from each farm. One to three fecal samples were collected from cows, and seven to nine environmental samples were collected from the dry-lot holding areas, manure spreaders, water trough areas, and various barn areas such as alleys and stalls.

Further studies were in a dairy herd of 25 cows in which the specified dry-cow treatment had not been used. Antibiotics, primarily penicillin and DSM, were used therapeutically for treatment of other conditions throughout the year. Records indicated limited usage. The herd was its own control.

Fecal material was studied because of the high concentration of *E. coli* and because transfer of resistance is more likely in the colon of animals than in the environment. To establish a baseline, one fecal sample was obtained.
from each cow before initiation of the dry-cow therapy program. To follow drug resistance in the enteric flora of the animals throughout the year without overburdening the laboratory, fecal specimens were collected from each cow just before the dry-cow treatment was administered and at 2 and 6 wk post-treatment. This procedure also permitted the assessment of treatment in the individual cow. To determine the influence of antibiotic drug usage and its subsequent selective pressure on incidence of drug resistance in the enteric flora of the animal population, the entire herd was sampled four times near the end of the year. They were sampled at 44, 46, 50, and 52 wk.

Environmental samples were collected from the manure spreader, barn cleaner, and lot area around the water tank. Ten samples were gathered from the three sources initially and every 2 wk through the year.

All samples were sent to the laboratory in sterile plastic bags. One-gram aliquots of the material were homogenized in appropriate amounts of buffered water and further diluted as necessary before plating on MacConkey agar, both plain and supplemented with DSM (25 \( \mu \text{g/ml} \)). The incidence of DSM-resistant organisms was determined by comparing colony counts of \( E. \text{coli} \) on each medium. Typical \( E. \text{coli} \) colonies were streaked onto fresh MacConkey agar plates from unsupplemented plates, and purified cultures were further identified on Triple Sugar Iron agar and Simmon's Citrate agar.

Antimicrobial sensitivity patterns were determined on individual \( E. \text{coli} \) isolates by the standardized single disk method (1). Antibiotics tested were ampicillin, cephalothin, sulfamethoxypyridazine, colistin, chloramphenicol, furazolidone (100 \( \mu \text{g} \)) (in place of nitrofurantoin), neomycin, polymyxin B, tetracycline, nalidixic acid, and DSM (10 \( \mu \text{g} \)) (in place of streptomycin). Minimal inhibitory concentrations (MIC's) of DSM were determined by a serial dilution microtechnique (4).

**Results**

During preliminary studies 47 and 40 isolates of \( E. \text{coli} \) were studied from control farms and from farms using the dry-cow treatment program, respectively. In both groups 10% of the isolates were resistant to DSM and/or ampicillin. Fifty percent or more of the isolates were sensitive to all drugs tested. The frequency of the other resistances was less than 10%.

Fecal samples from 23 cows were examined initially during October to determine a baseline (6.4%) for the incidence of \( E. \text{coli} \) resistant to DSM. This varied thereafter at 10% or
less in cows just before treatment or at 2 or 6 wk post-treatment (Fig. 1). Dry-cow treatment was administered to 13 cows during the first 8 wk, and further treatments were not administered until the 36th wk. Increases in the incidence of resistant organisms above the baseline were small in relation to administration of dry-cow treatments. However, peaks occurred at 44 and 46 wk when the entire herd was sampled. When the entire herd was sampled again at 50 and 52 wk, the incidence of DSM-resistant \( E. \) coli had dropped to below the baseline.

Change in the frequency of \( E. \) coli resistant to DSM (25 \( \mu g/ml \)) at 2 and 6 wk post-treatment was small compared with determinations just before treatment. Incidence of DSM-resistant organisms was between .2 and 10.0\% for most cows for all three sampling times. The number of cows with less than .2\% DSM-resistant organisms increased at 2 wk post-treatment. Two cows had baseline values greater than 10\% (11 and 54\%) and one had 12\% DSM-resistant organisms at 6 wk post-treatment. The mean incidence of DSM-resistant enteric organisms at 2 and 6 wk post-treatment was 1.0 and 2.0\%, versus the 6.0\% just before treatment. At 2 wk post-treatment, a decrease in frequency of DSM-resistant organisms occurred in 21 cows and an increase occurred in 6. At 6 wk post-treatment, the incidence of DSM-resistant \( E. \) coli had decreased in 16 animals and increased in 7. The largest decrease was 8.0\%. The largest increase was 12.0\%. Excluded was the one high baseline value of 54\% which had dropped to an undetectable level by 6 wk.

In the environmental samples, peak incidences of \( E. \) coli resistant to DSM occurred during the fall and again in late spring and summer (Fig. 2). These peaks corresponded to times when the dry-cow treatment was being administered. However, none of the three peaks was above 10\%. During the cold months of winter and early spring, total numbers of organisms in many of the environmental samples were quite low, as was the percentage of organisms resistant to DSM. The incidence of DSM resistance was below 1.0\% during the cold months, but these values were considered less reliable because few samples yielded results. Environmental samples were examined for effects of hydration on total \( E. \) coli counts and incidence of resistance; both total counts and resistant \( E. \) coli were independent of this factor.

![Graph showing incidence of \( Escherichia \) coli resistant to dihydrostreptomycin in the environment of cows treated by intramammary infusion of 1,000,000 units of penicillin and 1 g of dihydrostreptomycin.](image)
The number of samples having DSM-resistant E. coli and the incidence of resistance in these samples were similar for both the animal and environment categories (Table 1). Most samples contained less than 10% DSM-resistant organisms (88 and 96% of the samples for animal and environmental samples, respectively), and the majority of these were 1% or less.

Approximately 12% of E. coli isolates from fecal samples had single or multiple antimicrobial resistance patterns, as determined by the single disk technique. Eighty-one percent of these had an R+ determinant for DSM. Similarly, 8% of the E. coli isolates from environmental sources were singly or multiply resistant, and DSM resistance appeared in 54% of these isolates. The most common resistance patterns are in Table 2.

The MIC’s for DSM ranged from 5 to >2,500 μg/ml (Table 3). The frequency of E. coli isolates in each MIC range remained rather constant throughout the test period for both fecal and environmental samples.

**Discussion**

Characteristics of resistant organisms (Figs. 1 and 2) from both fecal and environmental samples were similar. Considering the source of environmental samples, with cows representing the major contributor of E. coli to the immediate environment, these results are logical. It would be difficult to determine if the persistently low resistant E. coli in the environment during winter months was due to weather effects or to lack of treatment in the herd. However, several therapeutic regimens of antibiotics (other than dry-cow treatments) were administered either by intramammary infusion or parenterally during this period and apparently resulted in no detectable effects.

The baseline for incidence of E. coli resistant to DSM was determined from one set of samples. Therefore, day-to-day variation expected in such a dynamic situation could not be determined. Previous work has shown enough day-to-day variation in levels of resistant enteric organisms in individual animals to suggest that daily sampling of animals would be advantageous when determining the effects of antimicrobials on levels of resistant enteric organisms (11). Peak incidences of resistant organisms in this experiment were relatively close to the baseline (10% compared with 6% for fecal samples and 10% compared with 3% for environmental samples). The test herd was not maintained under isolated conditions that would prevent introduction of drug-resistant

**Table 1.** Incidence of *Escherichia coli* resistant to dihydrostreptomycin (DSM) (25 μg/ml) in fecal samples from cows and environmental samples.

<table>
<thead>
<tr>
<th>Percentage of DSM-resistant E. coli</th>
<th>Fecal samples</th>
<th>Environmental samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>77 (46)</td>
<td>136 (68)</td>
</tr>
<tr>
<td>1-10</td>
<td>71 (42)</td>
<td>57 (28)</td>
</tr>
<tr>
<td>10-50</td>
<td>16 (9.5)</td>
<td>5 (2.5)</td>
</tr>
<tr>
<td>20-50</td>
<td>2 (1.2)</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>2 (1.2)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>201</td>
</tr>
</tbody>
</table>

**Table 2.** Most common resistance patterns in *Escherichia coli* isolates from fecal samples from cows and environmental samples.

<table>
<thead>
<tr>
<th>Pattern*</th>
<th>Source of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal</td>
<td>Environmental</td>
</tr>
<tr>
<td>DSM, TE</td>
<td>14</td>
</tr>
<tr>
<td>DSM, SU, TE</td>
<td>5</td>
</tr>
<tr>
<td>AM, DSM, SU, N, TE</td>
<td>4</td>
</tr>
<tr>
<td>AM, DSM, N, TE</td>
<td>3</td>
</tr>
<tr>
<td>AM, DSM, TE</td>
<td>3</td>
</tr>
<tr>
<td>AM</td>
<td>3</td>
</tr>
<tr>
<td>FX, TE</td>
<td>0</td>
</tr>
</tbody>
</table>

*Abbreviations: AM, ampicillin; DSM, dihydrostreptomycin; SU, sulfamethoxypyridazine; N, neomycin; TE, tetracycline; FX, furazolidone.

**Table 3.** Minimal inhibitory concentrations (MIC) of dihydrostreptomycin for *Escherichia coli* isolates.

<table>
<thead>
<tr>
<th>Source of isolate</th>
<th>Time of sampling</th>
<th>Number of E. coli isolates in MIC (μg/ml) range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal samples</td>
<td>Baseline</td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td>Day of treatment</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>2 wk post-treatment</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>6 wk post-treatment</td>
<td>26</td>
</tr>
<tr>
<td>Environmental samples</td>
<td>Baseline</td>
<td>159</td>
</tr>
</tbody>
</table>
organisms. Resistant bacteria from other sources, such as neighboring swine, poultry, or beef cattle herds that were given antimicrobial drugs in feeds, could have contributed to the test herd via movement on vehicles, farm implements, or boots. Therefore, the fluctuation in both the animal and environmental samples could have been due to normal variation and not to drug use in the herd.

In the model situation antibiotics are used therapeutically and are administered primarily by other than the oral route. In the dairy herd, large quantities (therapeutic levels) of antimicrobial drugs are usually administered one time (dry-cow treatment) or several times over a few days. Such usage in the test herd for 1 yr resulted in minimal effects on the incidence of resistant enteric or environmental \textit{E. coli}. Peak values were 10% or less and these rapidly decreased to a range of <.1% to approximately 2.0%. In contrast, oral use of antibiotics administered by capsule or in the diet in therapeutic or subtherapeutic amounts for short periods has resulted in 90 to 100% resistant \textit{E. coli} in animals (2, 3, 7). However, the extent and time of usage of antibiotics in these studies were limited, and the incidence of resistant organisms was decreased to or near the baseline value in days or a few weeks. Conversely, in animal herds where management practices utilize feeds supplemented with antimicrobials continually in most or all animals for most or all of their lives, the selective pressure may result in an incidence of resistant enteric organisms approaching 100%. This level has persisted for several months after administration of antibiotics has stopped (10).

By comparing the three general patterns of antibiotic usage (nonoral therapeutic use, limited feed use, or extensive feed use), speculation can be made about factors of each pattern which influence the "pool" of resistant organisms and its interaction with enteric flora. Animals can be expected to serve as major contributors of organisms to their immediate environment and, thus, serve as their own major source of organisms which are cycled through the gastrointestinal tract. For the dairy herd, a predominance of drug-sensitive \textit{E. coli} was shed primarily because selective pressure was not exerted by the oral use of antibiotics, and, therefore, a sensitive "pool" of organisms was maintained for cycling. Where the use of antibiotics in feed is unlimited, selective pressure causes a build-up of resistant organisms in the enteric flora of animals and consequently in the environment. When this pressure is removed, the enteric flora may revert to sensitive organisms, depending on the incidence of sensitive organisms in the environment. The environmental pool of organisms represents a source of organisms which either cycle through or colonize the gastrointestinal tract. As the environment becomes more saturated with resistant organisms via extensive pressure from continuous use of antibiotics, there is less chance of re-establishing a sensitive flora. Observations about this dynamic situation do not take into account differences in animal species and differences in ability of sensitive or resistant organisms to colonize the enteric tract. It is acknowledged that some species, like swine, have a higher degree of recycled organisms than do cattle, as would be expected because of differences in their eating habits.

**Conclusions**

In this dairy herd, therapeutic antibiotic treatment (intramammary use of large doses of penicillin and DSM) had little or no effect on drug resistance in \textit{E. coli}, in either the herd or its environment. These results contrast sharply to data from herds administered antibiotics continuously in the feed.

**Acknowledgments**

The authors thank Elaine Cohen and David Bray for their excellent technical assistance. The sample collection phase of this experiment was supported by Hamilton Pharmacal, Division of West Agro Chemicals, Inc., Long Island City, New York.

**References**


