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Article

# Frequency of Antimicrobial-Resistant Fecal *Escherichia coli* Among Small, Medium, and Large Beef Cow-Calf Operations in Florida

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## Simple Summary

Antimicrobial resistance (AMR) in beef cow-calf operations is an underexplored area of the beef industry, even though these operations are critical for supplying weaned calves to feedlots. In this study, we compared AMR in fecal bacteria from small, medium, and large beef cow-calf operations in Florida. We found that large beef cow-calf operations showed higher frequencies of resistance than medium and small operations, likely due to differences in antibiotic use and management practices. These findings provide new insights into the resistance patterns at the isolate and animal levels, as well as the differences between cows and calves, which may help researchers, veterinarians, and producers in developing strategies for monitoring and reducing AMR in beef cow-calf operations.

## Abstract

Antimicrobial resistance (AMR) is a growing threat to both animal and public health. This study describes the occurrence of AMR in *Escherichia coli* isolated from fecal samples from beef cow-calf operations and compares the frequency of antimicrobial-resistant fecal *E. coli* among small, medium, and large beef cow-calf operations in Florida, United States. This study was conducted using nine beef cow-calf operations. During farm visits conducted between December 2023 and April 2024, 743 fecal samples from cows (n=429) and calves (n=314) were collected either directly from the rectum or from fresh defecated feces. A total of 3,475 *E. coli* isolates (five isolates/animal) obtained from fecal samples of 695 cows and calves were selected for antimicrobial susceptibility testing. The antimicrobial susceptibility profile of each *E. coli* strain was determined by the Kirby-Bauer disk diffusion method. A panel of eight antibiotics was used to assess AMR in the fecal *E. coli* isolates. Irrespective of farm size, cows and calves showed higher resistance to streptomycin (47% and 30%), oxytetracycline (46% and 34%), sulfadimethoxine (42% and 27%), ampicillin (41% and 22%), and florfenicol (18% and 13%) at animal and isolate levels, respectively. In contrast, lower resistance frequencies were observed for gentamicin, ceftiofur, and trimethoprim/sulfamethoxazole, with values below 5% at the animal level, and approximately 1% at the isolate level. *E. coli* isolated from cows and calves showed higher resistance to one or more antibiotics in large beef cow-calf operations (66%) as compared to small (41%) and medium (53%) beef cow-calf operations (p < 0.0001). This study provides new data on AMR in fecal bacteria from beef cow-calf operations in Florida, which can help researchers, veterinarians, and producers develop strategies for monitoring and reducing AMR in these operations.

**Keywords:** Antibiotic resistance; *Escherichia coli*; Beef cow-calf operations; Operation size

## 1. Introduction

Antimicrobial resistance (AMR) is a significant problem in cattle production systems, because AMR pathogens can lead to increased morbidity and mortality in livestock, resulting in higher treatment and production costs for producers [1]. From a public health perspective, there is a risk that AMR pathogens may disseminate to humans either through direct contact with animals [2] or through the food chain [3,4]. The World Health Organization (WHO) has recognized AMR as one of the 21st century's biggest threats [5]. It has been estimated that, unless necessary measures are taken, AMR could cause up to ten million mortalities by 2050 [5]. Additionally, the economic loss associated with AMR is predicted to increase tremendously by the mid-21st century [5,6].

It is well established that the use of antibiotics is associated with an increase in AMR bacteria, but resistance can also develop in the absence of antibiotics [7]. Generic *Escherichia coli* is used as an indicator to monitor the changes in frequency and patterns of AMR, as these bacteria are components of the normal flora of the bovine gastrointestinal tract and a potential source for transmission of resistance genes to other bacteria [8,9].

On a global scale, there are significantly fewer studies investigating AMR levels in beef cow-calf operations [10]. Most studies investigating AMR have focused on feedlots, stockers, and slaughterhouses, where bacterial populations may experience elevated selection pressure owing to the use of antimicrobials for treatment or prevention. This pressure is often associated with management-related factors such as stress, transportation, and animal mixing [8].

In beef cow-calf operations, calves are born and remain with their dam cows at the same location until they are 6–8 months old. At that time, they were weaned, separated from their dam, and grouped with other animals of similar age and/or size. After weaning, most calves are transferred to feedlots, where they remain until they reach the slaughter weight. However, adult cull cows from cow-calf operations typically go straight to slaughter at the end of their productive life [8].

Florida is predominantly a cow-calf state and ranks tenth in beef cattle production, with approximately 865,000 heads of beef cows [11]. Moreover, very few studies have exclusively examined the AMR levels in beef cow-calf operations in Florida. However, understanding and characterizing AMR in these operations is crucial for evaluating the contribution of the cow-calf sector to AMR concerns in the beef industry. It is also important to understand whether specific management practices and patterns of antimicrobial use are associated with AMR during this stage, particularly before calves are moved to feedlots where higher selection pressures exist. Previous studies conducted in the western United States (California, Oregon, and Washington) and Florida indicated that management and operation-dependent factors, including operation size, can influence the presence of AMR [12,13]. Therefore, the main objectives of this study were to (i) compare the frequency of beef cattle harboring fecal *E. coli* resistant to selected antibiotics in small, medium, and large beef cow-calf operations; (ii) compare the frequency of *E. coli* isolates resistant to selected antibiotics from beef cattle in small, medium, or large beef cow-calf operations; and (iii) compare the frequency of cows and calves harboring fecal *E. coli* resistant to selected antibiotics in small, medium, or large cow-calf operations as a secondary objective.

## 2. Materials and Methods

### 2.1. Study Cow-Calf Operations

A convenience sample of nine beef cow-calf operations in Florida, USA, was enrolled in this study. Beef cow-calf operations were categorized based on the operation size: small (20-49 cows; n = 3), medium (50-199 cows; n = 3), and large ( $\geq 200$  cows; n = 3) [14]. These nine selected beef cow-calf operations represented standard management practices for small, medium, or large cow-calf operations in Florida. This study was approved by the IACUC (protocol # 202300000165) at the University of Florida.

2.2. Study Animals

A total of 97, 213, and 433 beef cattle (cows and calves) were selected from three small, three medium, and three large beef cow-calf operations on December 14, 2023, and April 4, 2024, respectively. The number of cattle from each study farm was justified using the following assumptions: the expected prevalence of AMR fecal *E. coli* was set at 50 ±10% for large farms, 25 ±10% for medium-sized farms, and 12.5 ±10% for small farms, with a confidence level of 95%. Prevalence rates of 50%, 25%, and 12.5% were based on a previous study [15]. Table 1 shows the number of animals included in each cow-calf operation.

**Table 1.** Number of cows and calves sampled and tested on each beef cow-calf operation.

| Beef cow-calf operation | Size   | Number of samples collected |        | Number of samples tested for AST |        |
|-------------------------|--------|-----------------------------|--------|----------------------------------|--------|
|                         |        | Cows                        | Calves | Cows                             | Calves |
| #1                      | Small  | 22                          | 11     | 20                               | 10     |
| #2                      |        | 18                          | 9      | 18                               | 9      |
| #3                      |        | 19                          | 18     | 18                               | 17     |
| #4                      | Medium | 48                          | 29     | 42                               | 26     |
| #5                      |        | 44                          | 30     | 40                               | 28     |
| #6                      |        | 32                          | 30     | 31                               | 28     |
| #7                      | Large  | 79                          | 62     | 76                               | 58     |
| #8                      |        | 82                          | 65     | 78                               | 61     |
| #9                      |        | 85                          | 60     | 80                               | 55     |
| Total                   |        | 429                         | 314    | 403                              | 292    |
|                         |        | 743                         |        | 695                              |        |

2.3. Collection of Fecal Samples

Fecal samples were collected directly from the rectum of each animal or from freshly defecated feces using individual rectal palpation sleeves or gloves to avoid contamination. Samples were collected in sterile bags, labeled, and stored on ice during transport to the laboratory at the University of Florida, College of Veterinary Medicine. The samples were processed and cultured within 24 h of collection to isolate the *E. coli*. Isolated *E. coli* were stored at -80 °C in 20% glycerol for subsequent analysis.

2.3. Isolation of Bacteria

All fresh fecal samples were directly plated onto *E. coli* ECD ChromoSelect agar with MUG (MilliporeSigma, Merck KGaA, Darmstadt, Germany) agar following the manufacturer's guidelines. Briefly, fecal samples were swabbed onto selective agar plates using a sterile cotton swab. The plates were incubated for 18-24 h at 44 °C, and *E. coli* colonies were identified based on their characteristic blue-green color. Colonies were confirmed by a positive indole spot test (Remel, Lenexa, KS, USA). Five discrete *E. coli* colonies from each sample plate were subcultured on MacConkey agar (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) and incubated at 37 °C for 24 h. Pink colonies from each plate were grown individually in Luria-Bertani (LB) broth at 37 °C and stored at -80°C in a mixture of LB broth and 25% glycerol in 2 ml cryovials until use.

2.4. *E. coli* Isolate Selection

After the initial culture of 743 fecal samples on ChromoSelect agar, we excluded 48 animal fecal samples because they either did not produce *E. coli* colonies or produced fewer than five discrete *E. coli* colonies. For antimicrobial susceptibility testing (AST), 3,475 isolates (five isolates/animal) obtained from 695 cow and calf fecal samples were selected. Table 1 lists the numbers of cows and calves selected for AST.

2.5. Antimicrobial Susceptibility Testing (AST)

The Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of *E. coli* [16]. Each isolate was tested against a panel of eight antibiotics. The antibiotics tested were penicillins (ampicillin, 10 µg), third-generation cephalosporins (ceftiofur, 30 µg), amphenicols (florfenicol, 30 µg), aminoglycosides (gentamicin, 10 µg; streptomycin, 10 µg), tetracyclines (oxytetracycline, 30 µg), trimethoprim-sulfonamide combination (trimethoprim/sulfamethoxazole, 1.25/23.75 µg), and sulfonamides (sulfadimethoxine, 300 µg). Antibiotic discs of standard concentrations were prepared in our laboratory, except for ceftiofur, as previously described [17]. Commercially available disks were used for ceftiofur (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA). The diameter of the zone of inhibition was measured using a Vernier caliper and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [18] or based on previous publications, if guidelines were not available [19]. *E. coli* ATCC® 25922 and *E. coli* ATCC® 35218 served as the reference strains. A cow/calf was classified as resistant (R) if one isolate out of five was resistant to at least one antibiotic and as multi-antibiotic resistant (MAR) if at least one isolate was resistant to  $\geq 3$  classes of antibiotics [20].

## 2.6. Data Analysis

A descriptive analysis was performed to determine the frequency (%) of antimicrobial-resistant *E. coli* at both the animal and isolate levels, using Microsoft Excel. The following null hypotheses were tested.

H<sub>01</sub>: There is no difference in the frequency of beef cattle with *E. coli* resistance to selected antibiotics in small, medium, and large beef cow-calf operations.

H<sub>02</sub>: There is no difference in the frequency of *E. coli* isolates with resistance in small, medium, and large beef cow-calf operations.

H<sub>03</sub>: There is no difference in the frequency of cows and calves diagnosed with *E. coli* resistance to selected antibiotics in small, medium, and large beef cow-calf operations.

Resistance frequencies between the groups were compared using the chi-square test, and a p-value of  $< 0.05$  was considered statistically significant. All Chi-square analyses were performed using MedCalc® statistical software.

## 3. Results

### 3.1. Isolation of *Escherichia coli* from fecal samples

*E. coli* was recovered from 94% (695/743) of cows and calves across nine beef cow-calf operations. A total of 3475 *E. coli* isolates from cows and calves were subjected to AST.

### 3.2. Overall Frequency of Antimicrobial Resistance

The frequency of AMR was determined at both the animal and isolate levels. Overall, resistance to one or more antibiotics was recorded in 59% (412/695) of the animals (cows and calves) and in 46% (1586/3475) of the isolates. 44% (304/695) of the animals and 24% (835/3475) of the isolates exhibited multi-antibiotic resistance (MAR). At the animal level, a higher proportion of cows and calves showed resistance to streptomycin (47% or 330/695), followed by oxytetracycline (46% or 318/695), sulfadimethoxine (42% or 291/695), ampicillin (41% or 283/695), and florfenicol (18% or 126/695). The lowest resistance was observed against gentamicin (4%, or 27/695), ceftiofur 3%, or 19/695), and trimethoprim/sulfamethoxazole (3%, or 18/695). A similar pattern emerged at the isolate level, where 34% (1166/3475) of the isolates were resistant to oxytetracycline, followed by streptomycin (30%, or 1041/3475), sulfadimethoxine (27%, or 955/3475), ampicillin (22%, or 777/3475), and florfenicol (13%, or 445/3475). The lowest resistance was observed against gentamicin (1%, or 33/3475), ceftiofur (1%, or 24/3475), and trimethoprim/sulfamethoxazole (1%, or 37/3475).

### 3.3. Comparison of AMR Across Farm Sizes at Animal Level

At the animal level, ampicillin resistance in fecal *E. coli* was observed in similar proportions across all operation sizes: 38% (35/92) in small operations, 38% (74/195) in medium operations, and

43% (175/408) in large operations, with no significant differences among the groups ( $p > 0.05$ ). Fecal *E. coli* resistant to ceftiofur was 0% (0/92) in animals from small beef cow-calf operations, 2% (4/195) in medium beef cow-calf operations, and 4% (15/408) in large operations, showing no statistically significant ( $p > 0.05$ ) difference among groups against ceftiofur as well. Gentamicin resistance was observed in 9% (8/92) of cows and calves from small operations, 4% (8/195) from medium operations, and 3% (11/408) from large operations. Although the overall comparison among these groups was statistically significant ( $p < 0.05$ ), only the comparison between large and small operations showed a statistically significant difference. Resistance to florfenicol in fecal *E. coli* was observed in 5% (5/92) of animals from small operations, 6% (12/195) from medium operations, and 27% (109/408) from large operations. The overall difference between the groups was statistically significant ( $p < 0.0001$ ). Pairwise comparisons revealed significant differences between small and large operations as well as between medium and large operations, while the difference between small and medium was not statistically significant ( $p > 0.05$ ). Fecal *E. coli* resistant to streptomycin was present in 58% (235/408) of animals from small operations, 37 (72/195) from medium operations, and 25% (23/92) from large operations. This indicated that the overall comparison and all pairwise comparisons for streptomycin resistance across the different farm sizes were statistically significant ( $p < 0.0001$ ). Resistance to oxytetracycline was observed in 53% (215/408) of animals from large operations, 39% (77/195) from medium operations, and 28% (26/92) from small beef cow-calf operations, with overall differences among the groups being statistically significant ( $p < 0.0001$ ). Pairwise comparisons showed that the difference between small and medium operations was not statistically significant ( $p > 0.05$ ), whereas the differences between small and large operations, as well as between medium and large operations, were statistically significant. Resistance to sulfadimethoxine was observed in 51% (209/408) of animals from large operations, 31% (61/195) from medium operations, and 23% (21/92) from small beef cow-calf operations. The overall difference among the groups was statistically significant ( $p < 0.0001$ ), whereas pairwise comparisons indicated that the difference between small and medium operations was not statistically significant ( $p > 0.05$ ), whereas the differences between small and large operations, as well as between medium and large operations, were significant ( $p < 0.0001$ ). Resistance to trimethoprim/sulfamethoxazole was observed in only 1% (5/408) of the animals from large operations, 6% (11/195) from medium operations, and 2% (2/92) from large operations. The overall differences between the groups were statistically significant ( $p < 0.05$ ). Pairwise comparisons showed no significant difference between small and medium operations, and small vs. large, but medium vs. large operations differed significantly. At the animal level, fecal *E. coli* from a significantly higher proportion of cows and calves from large beef cow-calf operations (66%, or 270/408) carried resistance to one or more antibiotics compared to those from medium-sized operations (53%, or 104/195) and small operations (41% (38/92) ( $p < 0.0001$ ). Pairwise comparisons revealed no significant difference between small and medium operations ( $p = 0.057$ ), whereas both small and large operations ( $p < 0.0001$ ) and medium vs. large operations ( $p = 0.002$ ) showed statistically significant differences. Similarly, MAR was more common among cows and calves from large operations (51%, or 208/408) than medium (34%, or 67/195) and small operations (32%, or 29/92), with a p-value of  $< 0.0001$ . While no significant difference was found between small and medium operations ( $p = 0.635$ ), the comparisons between small and large operations, as well as between medium and large operations, were statistically significant ( $p = 0.0007$  and  $p = 0.0001$ , respectively). Table 2 presents the results of AST at the animal level.

**Table 2.** Frequency of fecal *Escherichia coli* resistance to a panel of eight antibiotics in beef cow-calf operations (animal level).

|            | Small Cow-calf operations |               |               | Medium Cow-calf operations |               |               | Large Cow-calf operations |                |                | p-value |
|------------|---------------------------|---------------|---------------|----------------------------|---------------|---------------|---------------------------|----------------|----------------|---------|
|            | #1<br>n/N (%)             | #2<br>n/N (%) | #3<br>n/N (%) | #4<br>n/N (%)              | #5<br>n/N (%) | #6<br>n/N (%) | #7<br>n/N (%)             | #8<br>n/N (%)  | #9<br>n/N (%)  |         |
| Ampicillin | 12/30<br>(40)             | 8/27 (30)     | 14/35<br>(40) | 31/68<br>(46)              | 22/68<br>(32) | 21/59<br>(36) | 57/134<br>(43)            | 77/139<br>(55) | 41/135<br>(30) | 0.431   |
|            | 35/92 (38) <sup>a</sup>   |               |               | 74/195 (38) <sup>a</sup>   |               |               | 175/408 (43) <sup>a</sup> |                |                |         |

|                                   |                         |               |               |                           |               |               |                           |                 |                |         |
|-----------------------------------|-------------------------|---------------|---------------|---------------------------|---------------|---------------|---------------------------|-----------------|----------------|---------|
| Ceftiofur                         | 0/30<br>(0)             | 0/27<br>(0)   | 0/35<br>(0)   | 0/68<br>(0)               | 4/68<br>(6)   | 0/59<br>(0)   | 1/134<br>(1)              | 13/139<br>(9)   | 1/135<br>(1)   | 0.117   |
|                                   | 0/92 (0) <sup>a</sup>   |               |               | 4/195 (2) <sup>a</sup>    |               |               | 15/408 (4) <sup>a</sup>   |                 |                |         |
| Gentamicin                        | 4/30<br>(13)            | 2/27<br>(7)   | 2/35<br>(6)   | 2/68<br>(3)               | 4/68<br>(6)   | 2/59<br>(3)   | 7/134<br>(5)              | 2/139<br>(1)    | 2/135<br>(1)   | 0.026   |
|                                   | 8/92 (9) <sup>a</sup>   |               |               | 8/195 (4) <sup>ab</sup>   |               |               | 11/408 (3) <sup>b</sup>   |                 |                |         |
| Florfenicol                       | 2/30<br>(7)             | 1/27<br>(4)   | 2/35<br>(6)   | 1/68<br>(1)               | 0/68<br>(0)   | 11/59<br>(19) | 15/134<br>(11)            | 38/139<br>(27)  | 56/135<br>(41) | <0.0001 |
|                                   | 5/92 (5) <sup>a</sup>   |               |               | 12/195 (6) <sup>a</sup>   |               |               | 109/408 (27) <sup>b</sup> |                 |                |         |
| Streptomycin                      | 9/30<br>(30)            | 7/27 (26)     | 7/35<br>(20)  | 18/68<br>(26)             | 24/68<br>(35) | 30/59<br>(51) | 79/134<br>(59)            | 86/139<br>(62)  | 70/135<br>(52) | <0.0001 |
|                                   | 23/92 (25) <sup>a</sup> |               |               | 72/195 (37) <sup>b</sup>  |               |               | 235/408 (58) <sup>c</sup> |                 |                |         |
| Oxytetracycline                   | 10/30<br>(37)           | 9/27 (33)     | 7/35<br>(20)  | 18/68<br>(26)             | 24/68<br>(35) | 35/59<br>(59) | 54/134<br>(40)            | 92/139<br>(66)  | 69/135<br>(51) | <0.0001 |
|                                   | 26/92 (28) <sup>a</sup> |               |               | 77/195 (39) <sup>a</sup>  |               |               | 215/408 (53) <sup>b</sup> |                 |                |         |
| Sulfadimethoxine                  | 8/30<br>(27)            | 6/27 (22)     | 7/35<br>(20)  | 21/68<br>(31)             | 16/68<br>(24) | 24/59<br>(41) | 64/134<br>(48)            | 79/139<br>(57)  | 66/135<br>(49) | <0.0001 |
|                                   | 21/92 (23) <sup>a</sup> |               |               | 61/195 (31) <sup>a</sup>  |               |               | 209/408 (51) <sup>b</sup> |                 |                |         |
| Trimethoprim/<br>Sulfamethoxazole | 2/30<br>(7)             | 0/27<br>(0)   | 0/35<br>(0)   | 1/68<br>(1)               | 2/68<br>(3)   | 8/59<br>(14)  | 2/134<br>(1)              | 1/139<br>(1)    | 2/135<br>(1)   | 0.005   |
|                                   | 2/92 (2) <sup>a</sup>   |               |               | 11/195 (6) <sup>ab</sup>  |               |               | 5/408 (1) <sup>a</sup>    |                 |                |         |
| Resistant to ≥ 1                  | 13/30<br>(43)           | 11/27<br>(41) | 14/35<br>(40) | 33/68<br>(49)             | 36/68<br>(53) | 35/59<br>(59) | 91/134<br>(68)            | 105/139<br>(76) | 74/135<br>(55) | <0.0001 |
|                                   | 38/92 (41) <sup>a</sup> |               |               | 104/195 (53) <sup>a</sup> |               |               | 270/408 (66) <sup>b</sup> |                 |                |         |
| Multi resistant ≥3                | 13/30<br>(43)           | 7/27 (26)     | 9/35<br>(25)  | 18/68<br>(26)             | 21/68<br>(31) | 28/59<br>(47) | 63/134<br>(47)            | 78/139<br>(56)  | 67/135<br>(50) | <0.0001 |
|                                   | 29/92 (32) <sup>a</sup> |               |               | 67/195 (34) <sup>a</sup>  |               |               | 208/408 (51) <sup>b</sup> |                 |                |         |

Different superscript letters (a, b, c) within the same row indicate statistically significant differences (p < 0.05).

3.4. Comparison of AMR Across Farm Sizes at Isolate Level

Ampicillin resistance was detected in 27% (122/460) of the isolates from small operations, 24% (236/975) from medium operations, and 21% (419/2020) from large operations (p = 0.005). Pairwise comparisons indicated no significant difference between small and medium operations, whereas both small and large operations and medium vs. large operations were statistically significant. Resistance to ceftiofur was absent in isolates from small operations (0%, or 0/460), but was present in 0.4% (4/975) of isolates from medium operations, and 1% (20/2040) of isolates from large operations. Although the overall differences were statistically significant, pairwise analysis showed that only the small vs. large comparisons reached statistical significance (p = 0.033). Gentamicin resistance was low across all groups: 2% (8/460) in small operations, 1% (8/975) in medium operations, and 1% (17/2040) in large operations. The overall and pairwise differences were not statistically significant (p > 0.05). At the isolate level, florfenicol resistance was observed in 1% (5/460) of isolates from small operations, 4% (43/975) from medium operations, and 19% (397/2040) from large operations. Streptomycin resistance occurred in 37% (754/2040) of isolates from small operations, 22% (216/975) from medium operations, and 15% (71/460) from large operations. Oxytetracycline resistance was detected in 38% (782/2040) of isolates from large operations, 29% (278/975) from medium operations, and 23% (106/460) from small operations. Sulfadimethoxine resistance was recorded in 33% (670/2040) of the isolates from large operations, 24% (236/975) from medium operations, and 11% (49/460) from small operations. For florfenicol, streptomycin, oxytetracycline, and sulfadimethoxine, the overall differences among the small, medium, and large operations were statistically significant, and all pairwise comparisons between the operation sizes were also significant (p < 0.05). Trimethoprim/sulfamethoxazole resistance was rare, occurring in 0.4% (8/2040) of isolates from large operations, 3% (27/975) from medium operations, and 0.4% (2/460) from small operations. The overall comparison was statistically significant (p < 0.05); however, a significant difference was observed only between the small and medium operations. When considering resistance to at least one antibiotic, fecal *E. coli* from large beef

cow-calf operations showed the highest resistance, 48% (971/2040), followed by medium 45% (443/975), and small beef cow-calf operations 37% (172/460) ( $p = 0.004$ ). Pairwise comparisons showed significant differences between small and large operations ( $p = 0.0001$ ), as well as between small and medium operations ( $p = 0.004$ ). The difference between medium and large operations was not statistically significant ( $p = 0.265$ ). MAR was more frequent ( $p < 0.0001$ ) in fecal *E. coli* from large operations (31% or 623/2040) than in medium (16% or 155/975) or small (13% or 57/460) operations. However, no significant difference was found between small and medium operations. The comparisons between small and large operations, as well as between medium and large operations, were statistically significant. Table 3 presents the results of AST at the animal level.

**Table 3.** Frequency of fecal *Escherichia coli* resistance to a panel of eight antibiotics in beef cow-calf operations (isolate level).

|                                   | Small Cow-calf operations |                |                | Medium Cow-calf operations |                 |                 | Large Cow-calf operations  |                 |                 | p-value |
|-----------------------------------|---------------------------|----------------|----------------|----------------------------|-----------------|-----------------|----------------------------|-----------------|-----------------|---------|
|                                   | #1<br>n/N (%)             | #2<br>n/N (%)  | #3<br>n/N (%)  | #4<br>n/N (%)              | #5<br>n/N (%)   | #6<br>n/N (%)   | #7<br>n/N (%)              | #8<br>n/N (%)   | #9<br>n/N (%)   |         |
| Ampicillin                        | 44/150<br>(29)            | 20/135<br>(15) | 58/175<br>(33) | 102/340<br>(30)            | 61/340<br>(18)  | 73/295<br>(25)  | 112/670<br>(17)            | 223/695<br>(32) | 84/675<br>(12)  | 0.005   |
|                                   | 122/460 (27) <sup>a</sup> |                |                | 236/975 (24) <sup>a</sup>  |                 |                 | 419/2040 (21) <sup>b</sup> |                 |                 |         |
| Ceftiofur                         | 0/150<br>(0)              | 0/135<br>(0)   | 0/175<br>(0)   | 0/340<br>(0)               | 4/340<br>(1)    | 0/295<br>(0)    | 1/670<br>(0.1)             | 18/695<br>(3)   | 1/675<br>(0.1)  | 0.033   |
|                                   | 0/460 (0) <sup>a</sup>    |                |                | 4/975 (0.4) <sup>ab</sup>  |                 |                 | 20/2040 (1) <sup>b</sup>   |                 |                 |         |
| Gentamicin                        | 4/150<br>(3)              | 2/135<br>(1)   | 2/175<br>(1)   | 2/340<br>(1)               | 4/340<br>(1)    | 2/295<br>(1)    | 12/670<br>(2)              | 4/695<br>(1)    | 1/675<br>(0.3)  | 0.172   |
|                                   | 8/460 (2) <sup>a</sup>    |                |                | 8/975 (1) <sup>a</sup>     |                 |                 | 17/2040 (1) <sup>a</sup>   |                 |                 |         |
| Florfenicol                       | 2/150<br>(1)              | 1/135<br>(1)   | 2/175<br>(1)   | 1/340<br>(0.3)             | 0/340<br>(0)    | 42/295<br>(14)  | 40/670<br>(6)              | 143/695<br>(21) | 214/675<br>(32) | <0.0001 |
|                                   | 5/460 (1) <sup>a</sup>    |                |                | 43/975 (4) <sup>b</sup>    |                 |                 | 397/2040 (19) <sup>c</sup> |                 |                 |         |
| Streptomycin                      | 27/150<br>(18)            | 28/135<br>(21) | 16/175<br>(9)  | 37/340<br>(11)             | 69/340<br>(21)  | 110/295<br>(37) | 253/670<br>(38)            | 234/695<br>(34) | 267/675<br>(40) | <0.0001 |
|                                   | 71/460 (15) <sup>a</sup>  |                |                | 216/975 (22) <sup>b</sup>  |                 |                 | 754/2040 (37) <sup>c</sup> |                 |                 |         |
| Oxytetracycline                   | 42/150<br>(28)            | 43/135<br>(32) | 21/175<br>(12) | 62/340<br>(18)             | 67/340<br>(20)  | 149/295<br>(51) | 185/670<br>(28)            | 281/695<br>(40) | 316/675<br>(47) | <0.0001 |
|                                   | 106/460 (23) <sup>a</sup> |                |                | 278/975 (29) <sup>b</sup>  |                 |                 | 782/2040 (38) <sup>c</sup> |                 |                 |         |
| Sulfadimethoxine                  | 22/150<br>(15)            | 17/135<br>(13) | 9/175<br>(5)   | 32/340<br>(9)              | 34/340<br>(10)  | 82/295<br>(28)  | 196/670<br>(29)            | 219/695<br>(32) | 255/675<br>(38) | <0.0001 |
|                                   | 49/460 (11) <sup>a</sup>  |                |                | 236/975 (24) <sup>b</sup>  |                 |                 | 670/2040 (33) <sup>c</sup> |                 |                 |         |
| Trimethoprim/<br>Sulfamethoxazole | 2/150<br>(1)              | 0/135<br>(0)   | 0/175<br>(0)   | 1/340<br>(0.3)             | 7/340<br>(2)    | 19/295<br>(6)   | 2/670<br>(0.3)             | 2/695<br>(0.3)  | 4/675<br>(0.6)  | <0.0001 |
|                                   | 2/460 (0.4) <sup>a</sup>  |                |                | 27/975 (3) <sup>b</sup>    |                 |                 | 8/2040 (0.4) <sup>ab</sup> |                 |                 |         |
| Resistant to ≥ 1                  | 60/150<br>(40)            | 46/135<br>(34) | 66/175<br>(38) | 147/340<br>(43)            | 136/340<br>(40) | 160/295<br>(54) | 298/670<br>(44)            | 339/695<br>(49) | 334/675<br>(49) | 0.004   |
|                                   | 172/460 (37) <sup>a</sup> |                |                | 443/975 (45) <sup>b</sup>  |                 |                 | 971/2040 (48) <sup>b</sup> |                 |                 |         |
| Multi resistant ≥3                | 24/150<br>(16)            | 24/135<br>(18) | 10/175<br>(6)  | 28/340<br>(8)              | 30/340<br>(9)   | 97/295<br>(33)  | 162/670<br>(24)            | 200/695<br>(29) | 261/675<br>(39) | <0.0001 |
|                                   |                           |                |                |                            |                 |                 |                            |                 |                 |         |
|                                   | 57/460 (13) <sup>a</sup>  |                |                | 155/975 (16) <sup>a</sup>  |                 |                 | 623/2040 (31) <sup>b</sup> |                 |                 |         |

Different superscript letters (a, b, c) within the same row indicate statistically significant differences ( $p < 0.05$ ).

3.5. Comparison of AMR Between Cows and Calves

Table 4 presents a comparison of AMR between cows and calves across all nine beef cow-calf operations. Overall, a significantly higher proportion of cows, at 62% (250/403), exhibited resistance to one or more antibiotics ( $R > 1$ ) compared to calves, which showed a resistance of 52% (152/292) ( $p = 0.011$ ). While MAR was also more common in cows 46% (187/403) than in calves 40% (116/292), this difference was not statistically significant ( $p = 0.080$ ). When stratified by operation size,

- In small beef cow-calf operations, resistance to one or more antibiotics was observed in 41% (23/56) of cows and 42% (15/36) of calves, indicating similar resistance.
- In medium operations, resistance to one or more antibiotics was found in 50% (57/113) of the cows and 45% (37/82) of the calves.
- In large operations, resistance to one or more antibiotics was observed in 73% (170/234) of cows and 57% (100/174) of calves.

Although resistance to one or more antibiotics appeared to be more frequent in cows across all operation sizes, the differences between cows and calves within each operation size were not statistically significant, except in large beef cow-calf operations.

**Table 4.** Frequency of fecal *Escherichia coli* resistance to a panel of eight antibiotics in beef cow-calf operations (cows vs calves).

|         | Small Cow-calf operations |                      |                       |                       |                 |                       | Medium Cow-calf operations |                       |                       |                       |                       |                       | Large Cow-calf operations |                       |                       |                       |                       |                       |
|---------|---------------------------|----------------------|-----------------------|-----------------------|-----------------|-----------------------|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|         | Cows                      |                      |                       | Calves                |                 |                       | Cows                       |                       |                       | Calves                |                       |                       | Cows                      |                       |                       | Calves                |                       |                       |
|         | #1                        | #2                   | #3                    | #1                    | #2              | #3                    | #4                         | #5                    | #6                    | #4                    | #5                    | #6                    | #7                        | #8                    | #9                    | #7                    | #8                    | #9                    |
| A<br>MP | 8/2<br>0<br>(40)          | 5/<br>18<br>(2<br>8) | 7/1<br>8<br>(3<br>9)  | 4/1<br>0<br>(4<br>0)  | 3/9<br>(33<br>) | 7/1<br>7<br>(41<br>)  | 19/<br>42<br>(45<br>)      | 10/<br>40<br>(25<br>) | 10/<br>31<br>(32<br>) | 12/<br>26<br>(46<br>) | 12/<br>28<br>(43<br>) | 11/<br>28<br>(39<br>) | 33/<br>76<br>(43<br>)     | 49/<br>78<br>(63<br>) | 29/<br>80<br>(36<br>) | 24/<br>58<br>(41<br>) | 28/<br>61<br>(46<br>) | 12/<br>55<br>(22<br>) |
|         | 20/56 (36)                |                      |                       | 14/36 (39)            |                 |                       | 39/113 (35)                |                       |                       | 35/82 (43)            |                       |                       | 110/234 (47)              |                       |                       | 64/174 (37)           |                       |                       |
|         | 0/2<br>0<br>(0)           | 0/<br>18<br>(0)      | 0/1<br>8<br>(0.<br>0) | 0/1<br>0<br>(0.<br>0) | 0/9<br>(0)      | 0/1<br>7<br>(0)       | 0/4<br>2<br>(0)            | 1/4<br>0<br>(2.5<br>) | 0/3<br>1<br>(0)       | 0/2<br>6<br>(0)       | 3/2<br>8<br>(11<br>)  | 0/2<br>8<br>(0)       | 0/7<br>6<br>(0)           | 12/<br>78<br>(15<br>) | 1/8<br>0<br>(1)       | 1/5<br>8<br>(2)       | 1/6<br>1<br>(2)       | 0/5<br>5<br>(0)       |
| CE<br>F | 0/56 (0)                  |                      |                       | 0/36 (0)              |                 |                       | 1/113 (1)                  |                       |                       | 3/82 (4)              |                       |                       | 13/234 (6)                |                       |                       | 2/174 (1)             |                       |                       |
|         | 2/2<br>0<br>(10)          | 2/<br>18<br>(1<br>1) | 1/1<br>8<br>(6)       | 2/1<br>0<br>(2<br>0)  | 0/9<br>(0)      | 1/1<br>7<br>(6)       | 1/4<br>2<br>(2)            | 3/4<br>0<br>(8)       | 1/3<br>1<br>(3)       | 1/2<br>6<br>(4)       | 1/2<br>8<br>(4)       | 1/2<br>8<br>(4)       | 7/7<br>6<br>(9)           | 0/7<br>8<br>(0)       | 2/8<br>0<br>(3)       | 0/5<br>8<br>(0)       | 2/6<br>1<br>(3)       | 0/5<br>5<br>(0)       |
|         | 5/56 (9)                  |                      |                       | 3/36 (8)              |                 |                       | 5/113 (4)                  |                       |                       | 3/82 (4)              |                       |                       | 9/234 (4)                 |                       |                       | 2/174 (1)             |                       |                       |
| GE<br>N | 1/2<br>0<br>(5.0<br>0)    | 1/<br>18<br>(6)      | 2/1<br>8<br>(1<br>7)  | 1/1<br>0<br>(1<br>0)  | 0/9<br>(0)      | 0/1<br>7<br>(0.<br>0) | 1/4<br>2<br>(2)            | 0/4<br>0<br>(0)       | 6/3<br>1<br>(19<br>)  | 0/2<br>6<br>(0)       | 0/2<br>8<br>(0)       | 5/2<br>8<br>(18<br>)  | 10/<br>76<br>(13<br>)     | 37/<br>78<br>(47<br>) | 52/<br>80<br>(65<br>) | 5/5<br>8<br>(9)       | 1/6<br>1<br>(2)       | 4/5<br>5<br>(7)       |
|         | 4/56 (7)                  |                      |                       | 1/36 (3)              |                 |                       | 7/113 (6)                  |                       |                       | 5/82 (6)              |                       |                       | 99/234 (42)               |                       |                       | 10/174 (6)            |                       |                       |
|         | 6/2<br>0<br>(30)          | 5/<br>18<br>(2<br>8) | 5/1<br>8<br>(2<br>8)  | 3/1<br>0<br>(3<br>0)  | 2/9<br>(22<br>) | 2/1<br>7<br>(12<br>)  | 12/<br>42<br>(29<br>)      | 9/4<br>0<br>(23<br>)  | 16/<br>31<br>(52<br>) | 6/2<br>6<br>(23<br>)  | 15/<br>28<br>(54<br>) | 14/<br>28<br>(50<br>) | 47/<br>76<br>(62<br>)     | 55/<br>78<br>(71<br>) | 55/<br>80<br>(69<br>) | 32/<br>58<br>(55<br>) | 31/<br>61<br>(51<br>) | 15/<br>55<br>(27<br>) |
| FF<br>C | 16/56 (29)                |                      |                       | 7/36 (19)             |                 |                       | 37/113 (33)                |                       |                       | 35/82 (43)            |                       |                       | 157/234 (67)              |                       |                       | 78/174 (45)           |                       |                       |
|         | 6/2<br>0<br>(30)          | 7/<br>18<br>(3<br>9) | 0/1<br>8<br>(0)       | 5/1<br>0<br>(5<br>0)  | 2/9<br>(22<br>) | 7/1<br>7<br>(41<br>)  | 7/4<br>2<br>(17<br>)       | 10/<br>40<br>(25<br>) | 19/<br>31<br>(61<br>) | 11/<br>26<br>(42<br>) | 14/<br>28<br>(50<br>) | 16/<br>28<br>(57<br>) | 20/<br>76<br>(26<br>)     | 57/<br>78<br>(73<br>) | 56/<br>80<br>(70<br>) | 34/<br>58<br>(59<br>) | 35/<br>61<br>(57<br>) | 13/<br>55<br>(24<br>) |
|         | 13/56 (23)                |                      |                       | 14/36 (39)            |                 |                       | 36/113 (32)                |                       |                       | 41/82 (50)            |                       |                       | 133/234 (57)              |                       |                       | 82/174 (47)           |                       |                       |
| ST<br>R | 6/2<br>0<br>(30)          | 4/<br>18<br>(2<br>2) | 4/1<br>8<br>(4<br>4)  | 2/1<br>0<br>(2<br>0)  | 2/9<br>(22<br>) | 3/1<br>7<br>(18<br>)  | 12/<br>42<br>(29<br>)      | 8/4<br>0<br>(20<br>)  | 12/<br>31<br>(39<br>) | 9/2<br>6<br>(35<br>)  | 8/2<br>8<br>(29<br>)  | 12/<br>28<br>(43<br>) | 35/<br>76<br>(46<br>)     | 50/<br>78<br>(64<br>) | 54/<br>80<br>(68<br>) | 29/<br>58<br>(50<br>) | 29/<br>61<br>(48<br>) | 12/<br>55<br>(22<br>) |
|         | 14/56 (25)                |                      |                       | 7/36 (19)             |                 |                       | 32/113 (28)                |                       |                       | 29/82 (35)            |                       |                       | 139/234 (59)              |                       |                       | 70/174 (40)           |                       |                       |
|         | 1/2<br>0<br>(5)           | 0/<br>18<br>(0)      | 0/1<br>8<br>(0)       | 1/1<br>0<br>(1<br>0)  | 0/9<br>(0)      | 0/1<br>7<br>(0)       | 0/4<br>2<br>(0)            | 1/4<br>0<br>(3)       | 0/3<br>1<br>(0)       | 1/2<br>6<br>(4)       | 1/2<br>8<br>(4)       | 8/2<br>8<br>(29<br>)  | 2/7<br>6<br>(3)           | 1/7<br>8<br>(1)       | 1/8<br>0<br>(1)       | 0/5<br>8<br>(0)       | 0/6<br>1<br>(0)       | 1/5<br>5<br>(2)       |
| OT<br>C | 1/56 (2)                  |                      |                       | 1/36 (3)              |                 |                       | 1/113 (1)                  |                       |                       | 10/82 (12)            |                       |                       | 4/234 (2)                 |                       |                       | 1/174 (1)             |                       |                       |
|         | 8/2<br>0                  | 8/<br>18             | 7/1<br>8              | 5/1<br>0              | 3/9             | 7/1<br>7              | 20/<br>42                  | 18/<br>40             | 19/<br>31             | 13/<br>26             | 18/<br>28             | 16/<br>28             | 54/<br>76                 | 61/<br>78             | 55/<br>80             | 37/<br>58             | 44/<br>61             | 19/<br>55             |
|         |                           |                      |                       |                       |                 |                       |                            |                       |                       |                       |                       |                       |                           |                       |                       |                       |                       |                       |
| SD<br>M | 8/2<br>0                  | 8/<br>18             | 7/1<br>8              | 5/1<br>0              | 3/9             | 7/1<br>7              | 20/<br>42                  | 18/<br>40             | 19/<br>31             | 13/<br>26             | 18/<br>28             | 16/<br>28             | 54/<br>76                 | 61/<br>78             | 55/<br>80             | 37/<br>58             | 44/<br>61             | 19/<br>55             |
|         |                           |                      |                       |                       |                 |                       |                            |                       |                       |                       |                       |                       |                           |                       |                       |                       |                       |                       |
|         |                           |                      |                       |                       |                 |                       |                            |                       |                       |                       |                       |                       |                           |                       |                       |                       |                       |                       |
| SX<br>T | 1/2<br>0<br>(5)           | 0/<br>18<br>(0)      | 0/1<br>8<br>(0)       | 1/1<br>0<br>(1<br>0)  | 0/9<br>(0)      | 0/1<br>7<br>(0)       | 0/4<br>2<br>(0)            | 1/4<br>0<br>(3)       | 0/3<br>1<br>(0)       | 1/2<br>6<br>(4)       | 1/2<br>8<br>(4)       | 8/2<br>8<br>(29<br>)  | 2/7<br>6<br>(3)           | 1/7<br>8<br>(1)       | 1/8<br>0<br>(1)       | 0/5<br>8<br>(0)       | 0/6<br>1<br>(0)       | 1/5<br>5<br>(2)       |
|         | 1/56 (2)                  |                      |                       | 1/36 (3)              |                 |                       | 1/113 (1)                  |                       |                       | 10/82 (12)            |                       |                       | 4/234 (2)                 |                       |                       | 1/174 (1)             |                       |                       |
|         |                           |                      |                       |                       |                 |                       |                            |                       |                       |                       |                       |                       |                           |                       |                       |                       |                       |                       |
|         | 8/2<br>0                  | 8/<br>18             | 7/1<br>8              | 5/1<br>0              | 3/9             | 7/1<br>7              | 20/<br>42                  | 18/<br>40             | 19/<br>31             | 13/<br>26             | 18/<br>28             | 16/<br>28             | 54/<br>76                 | 61/<br>78             | 55/<br>80             | 37/<br>58             | 44/<br>61             | 19/<br>55             |
|         |                           |                      |                       |                       |                 |                       |                            |                       |                       |                       |                       |                       |                           |                       |                       |                       |                       |                       |
|         |                           |                      |                       |                       |                 |                       |                            |                       |                       |                       |                       |                       |                           |                       |                       |                       |                       |                       |

|               |                  |                      |                      |                      |                 |                      |                       |                      |                       |                      |                       |                       |                       |                       |                       |                       |                       |                       |
|---------------|------------------|----------------------|----------------------|----------------------|-----------------|----------------------|-----------------------|----------------------|-----------------------|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| R<br>to ≥     | (40)             | (4<br>4)             | (3<br>9)             | (5<br>0)             | (33<br>)        | (42<br>)             | (48<br>)              | (45<br>)             | (61<br>)              | (50<br>)             | (64<br>)              | (57<br>)              | (71<br>)              | (78<br>)              | (69<br>)              | (63<br>)              | (72<br>)              | (35<br>)              |
| 1             | 23/56 (41)       |                      |                      | 15/36 (42)           |                 |                      | 57/113 (50)           |                      |                       | 37/82 (45)           |                       |                       | 170/234 (73)          |                       |                       | 100/174 (57)          |                       |                       |
| M<br>DR<br>≥3 | 8/2<br>0<br>(40) | 5/<br>18<br>(2<br>7) | 5/1<br>8<br>(2<br>8) | 5/1<br>0<br>(5<br>0) | 2/9<br>(22<br>) | 4/1<br>7<br>(24<br>) | 10/<br>42<br>(24<br>) | 8/4<br>0<br>(20<br>) | 14/<br>31<br>(45<br>) | 8/2<br>6<br>(31<br>) | 12/<br>28<br>(43<br>) | 14/<br>28<br>(50<br>) | 32/<br>76<br>(42<br>) | 51/<br>78<br>(65<br>) | 54/<br>80<br>(68<br>) | 31/<br>58<br>(53<br>) | 27/<br>61<br>(44<br>) | 13/<br>55<br>(24<br>) |
|               | 18/56 (32)       |                      |                      | 11/36 (31)           |                 |                      | 32/113 (28)           |                      |                       | 34/82 (41)           |                       |                       | 137/234 (59)          |                       |                       | 71/174 (41)           |                       |                       |

R ≥ 1: Cows 250/403 (62%); Calves = 152/292 (52%) p-value 0.011, R ≥ 3: Cows 187/403 (46%); Calves = 116/292 (40%) p-value 0.080

4. Discussion

This study provides new information on the frequency and comparison of antimicrobial-resistant fecal *E. coli* across small, medium, and large beef cow-calf operations. It provides a detailed overview of differences by operation size at the animal level, isolate level, and cow vs. calf comparisons. To our knowledge, this is the first study to systematically compare AMR across different operation sizes. Using representative samples from nine beef cow-calf operations determined through sample size calculations, validated Kirby-Bauer disk diffusion testing, and rigorous statistical analyses, including pairwise comparisons, our results are robust and reliable and provide evidence-based information.

*E. coli* was recovered from 695 out of 743 fecal samples (94%), a recovery rate comparable to that reported in the National Animal Health Monitoring System (NAHMS) Beef 2017 study [21]. Overall, resistance to one or more antibiotics was observed in 59% of the animals (cows and calves) and in 46% of the *E. coli* isolates. An animal was classified as resistant if at least one *E. coli* isolate exhibited resistance to any of the antibiotics tested. This approach typically results in slightly higher proportions of resistance at the animal level than at the isolate level. Our findings showed a lower frequency of resistance than those of Gow et al. [22], who reported that 64.7% (134/207) of *E. coli* isolates were resistant to at least one antibiotic. However, results similar to those of our study were reported by Ferroni et al. [15] in Italy, with 41% of isolates resistant to at least one antibiotic, and by Morris et al. [23] in California, USA, where 36.1% of isolates showed resistance. In contrast, Fossen et al. [24] reported a much lower resistance rate of 16% in beef cow-calf operations in Canada.

Among the antibiotics tested, the highest resistance was observed to streptomycin (47% at the animal level and 30% at the isolate level), followed by oxytetracycline (46% at the animal level and 34% at the isolate level), sulfadimethoxine (42% at the animal level and 27% at the isolate level), ampicillin (41% at the animal level and 22% at the isolate level), and florfenicol (18% at the animal level and 13% at the isolate level). The resistance patterns observed in our study were similar to those reported by Morris et al. [23] in the USA and closely aligned with the findings of Gow et al. and Gow and Waldner [25,26] in Canadian cow-calf operations. Our results were also consistent with broader reports of AMR in *E. coli* from cattle. For instance, Massé et al. [27] reported resistance to tetracycline (26%), sulfisoxazole (23%), and streptomycin (19%) in dairy farms, whereas Veloo et al. [28] found the highest resistance in dairy farms to ampicillin (18.3%), followed by trimethoprim-sulfamethoxazole (8.9%). Similarly, Abdelfattah et al. [29] reported high levels of resistance to florfenicol, spectinomycin, oxytetracycline, sulphadimethoxine, and ampicillin in dairy cattle.

In our study, 46% of the animals and 34% of the isolates were resistant to oxytetracycline. The higher prevalence of oxytetracycline resistance compared to other antibiotics is likely due to the frequent use of long-acting oxytetracycline by ranchers for treating sick cows and calves in beef cow-calf operations. A higher percentage of operations used tetracyclines in feed for unweaned calves than for any other antibiotic [14]. Furthermore, a higher percentage of operations used tetracyclines in cows (18.4%) than the use of other antibiotics [14]. Markland et al. [13] also reported that oxytetracycline, a broad-spectrum tetracycline commonly used in feed to prevent diseases and infections, was the most commonly used antibiotic in beef cow-calf operations. Oxytetracycline and chlortetracycline also account for 42.2% of antibiotic sales in the livestock industry, making up the

largest portion of antibiotics, whereas tetracyclines represent only a very small fraction of the human medical industry at 3.9% [30]. Tetracycline resistance has consistently been reported as one of the most prevalent resistance patterns in *E. coli* isolated from beef cow-calf systems [7,15,25,26,31–33]. Similar resistance trends have also been reported in other sectors of the cattle industry, including dairy farms, with a prevalence of 70% [29] and 26% [27], and in feedlot farms, with a prevalence of 35.8% [34]. This widespread occurrence of tetracycline-resistant *E. coli* may be driven by the horizontal transfer of tetracycline-resistance genes among *E. coli* populations, sustained under selective pressure from the historic and ongoing use of tetracyclines in these systems [35].

Resistance to streptomycin was observed in 47% of animals and 30% of isolates in our study. This level of resistance was higher than that reported in other studies. For instance, Carson et al. [31] reported 7% resistance, while Waldner et al. [33] reported 3% (8/305) resistance in beef cow-calf operations. However, Gow et al. [22] and Gow et al. [26] reported much higher streptomycin resistance rates of 42% (86/207) and 67% (71/106), respectively. In another study, Gow et al. [25] reported 49% streptomycin resistance in spring-born calves and only 5% streptomycin resistance in fall-born calves. The elevated streptomycin resistance observed in our study may reflect past or undocumented use of aminoglycosides, such as streptomycin, in cow-calf operations or may result from cross-resistance associated with the use of other aminoglycosides. Additionally, although streptomycin is no longer widely used in contemporary veterinary practice, resistance may persist owing to co-selection mechanisms, where resistance genes are carried on mobile genetic elements alongside genes for other antimicrobials. However, due to the lack of detailed antimicrobial use data from participating farms, our ability to interpret these findings is limited.

In our study, the overall resistance to ampicillin was 41% at the animal level, and 22% at the isolate level. In comparison, Morris et al. [23] reported 100% (244/244) resistance at the isolate level in cow-calf operations in California, although none of the participating farms reported the use of ampicillin. In another study conducted in Italy on beef cow-calf herds, Ferroni et al. [15] reported 16% (33/212) resistance at the isolate level, and approximately 39% (21/54) at the farm level. Gow and Waldner [26] observed 32% (34/106) resistance, whereas Gow et al. [22] reported a lower frequency of 18% (38/207). Abdelfattah et al. [29] found 10% resistance to ampicillin at the cow level, and Veloo et al. [28] reported 18.3% resistance in *E. coli* isolates from dairy cattle. Although our study did not collect specific data on ampicillin use at the farm level, Markland et al. [13] documented the use of penicillin-class antibiotics in cow-calf operations in Florida. These findings suggest that resistance to ampicillin remains widespread and may persist due to past use, undocumented therapeutic applications, or co-selection with other  $\beta$ -lactam antibiotics.

In our study, resistance to sulfadimethoxine was observed in 42% of animals and 27% of isolates. These findings are consistent with previous reports; for example, Morris et al. [23] documented 25% (62/244) sulfadimethoxine resistance in beef cow-calf operations, whereas Abdelfattah et al. [29] reported 32% resistance in dairy cattle in California. The moderate to high levels of resistance across both the beef and dairy sectors may be attributed to the historical use of sulfonamides, such as sulfadimethoxine, sulfisoxazole, and sulfamethoxazole, either as therapeutic agents or for metaphylactic purposes. Although the use of sulphonamides has declined in recent years, resistance may persist in bacterial populations. Moreover, there is well-documented cross-resistance between sulfonamides, further complicating efforts to attribute resistance to a specific sulfa compound. The continued detection of resistance to sulfadimethoxine, even in the apparent absence of current use, underscores the importance of ongoing surveillance and the need to evaluate AMR through a One Health lens, considering environmental reservoirs and past antimicrobial practices.

In our study, resistance to ceftiofur, gentamicin, and trimethoprim/sulfamethoxazole was low, with frequencies of less than 5% at the animal level and approximately 1% at the isolate level. These findings align with those of previous research conducted on beef cow-calf operations. For instance, Morris et al. [23] reported 0.41% (1/244) resistance to ceftiofur and around 5% (12/244) resistance to trimethoprim/sulfamethoxazole at the isolate level. Similarly, Carson et al. [31] observed 2.5% resistance to trimethoprim/sulfamethoxazole. Gow and Waldner [26] reported resistance rates of

approximately 2% (2/106) to both ceftiofur and gentamicin, although a significantly higher resistance rate of 29% (31/106) was observed for trimethoprim/sulfamethoxazole. In contrast, Ferroni et al. [15] reported higher resistance rates among beef cow-calf herds in Italy, with 12% (25/212) at the isolate level and 32% (17/54) at the farm level for gentamicin, and 11% (23/212) at the isolate level and 35% (19/54) at the farm level for trimethoprim/sulfamethoxazole. Additionally, Abdelfattah et al. [29] reported that resistance in dairy cattle was 12.5% at the cow level and 2% at the isolate level for ceftiofur; 0.32% at the isolate level and 2.5% at the cow level for gentamicin; and 44% at the isolate level and 25% at the cow level for trimethoprim/sulfamethoxazole. These comparisons suggest that resistance to these antimicrobials remains relatively low in our study population, although substantial variation exists across regions, patterns of antimicrobial use, and production systems.

In the current study, the overall frequency of florfenicol resistance was 18% at the animal level and 13% at the isolate level. Resistance was notably higher in large cow-calf operations, with 19% and 27% resistance observed at the animal and isolate levels, respectively. This may be attributed to the reported use of florfenicol in these operations, particularly in the treatment of clinically ill animals. Farmers in large operations specifically noted the use of florfenicol as part of their treatment protocols, which may have contributed to increased selection pressure and subsequent resistance. Similar findings were reported by Morris et al. [23], who observed a florfenicol resistance frequency of approximately 19% in *E. coli* isolates from beef cow-calf operations. In a study conducted on dairy farms in the USA, Abdelfattah et al. [29] reported the highest *E. coli* resistance against florfenicol, with an isolate-level prevalence of 83.31%. An increasing trend of florfenicol resistance in *Enterobacteriaceae* has been reported in the literature, raising public health concerns because of its importance in human medicine. Florfenicol, a relatively newer broad-spectrum antibiotic, was first approved for use in cattle in 1996 [36]. It was introduced as a safer alternative to chloramphenicol, which has been banned in food-producing animals in several countries, including the United States and Canada, due to its association with aplastic anemia. It is normally used to treat keratoconjunctivitis [37], bacterial pneumonia, respiratory infections [38], and infectious pododermatitis [39]. Resistance to florfenicol is often mediated by mobile genetic elements such as plasmids and integrons, which facilitate horizontal gene transfer among bacterial populations. These mechanisms significantly enhance the dissemination of resistance within and between the microbial communities. Given the growing use of florfenicol in livestock and its potential to promote resistance, future research should focus on understanding the genetic mechanisms and risk factors driving this trend, particularly in large-scale production systems.

In our study, we found that resistance to at least one antibiotic was generally higher in large beef cow-calf operations than in medium and small operations. This trend was also observed for MAR, with large operations exhibiting the highest MAR levels. Resistance to many antibiotics, such as streptomycin, oxytetracycline, sulfadimethoxine, and florfenicol, was notably higher in large operations. However, small operations exhibited higher resistance to gentamicin and trimethoprim/sulfamethoxazole at both animal and isolate levels. Ampicillin resistance deviated from this general trend, showing higher levels in small operations at the isolate level but higher levels in large operations at the animal level. These findings are consistent with those of Markland et al. [13] and Ferroni et al. [15], who also reported a higher AMR in large-scale farms. However, these studies did not explain why large beef cow-calf operations exhibited more resistance. Possible explanations include a higher antimicrobial selective pressure due to the increased use of antibiotics. In 2017, 79.8% of medium-sized and 82.9% of large beef cow-calf operations used oral or injectable antibiotics for treatment compared to only 42.1% in small operations [14]. In addition to antibiotic use, other management factors, such as population density, farm hygiene, stress, calving season, and biosecurity practices, may contribute to the higher AMR observed in large operations. However, these factors were not measured in our study; therefore, the attribution of resistance to these factors remains speculative.

This study was not specifically designed to compare AMR between cows and calves; however, we observed that overall resistance to at least one antibiotic was higher in cows than in calves.

Notably, this difference was statistically significant only in large cow-calf operations. One possible explanation is the higher likelihood of antimicrobial use in cows for therapeutic or prophylactic purposes in large operations, potentially contributing to the increased selection pressure. Although MAR was also more frequent in cows than in calves, the difference was not statistically significant. These findings differ from those of previous studies, which reported a higher prevalence of AMR in calves than in adult cattle [12,34]. However, it is important to note that calves in those studies were typically less than four weeks old, whereas in our study, calves were up to six to eight months old. The bacterial AMR profile in neonates likely differs from that in older calves, which may explain this discrepancy. Additionally, in dairy systems, young calves often receive antibiotic treatments more frequently to manage neonatal diseases, which may not be the case in beef cow-calf operations. In beef cow-calf systems, antimicrobial use patterns and disease risk differ, potentially reducing the exposure of beef calves to antibiotics.

Our study was designed to test three hypotheses regarding AMR in beef cow-calf operations. The results support all the three hypotheses. At the animal and isolate levels, the resistance to one or more antibiotics and MAR was higher in large beef cow-calf operations than in medium and small operations. AMR in cows was more resistant than that in calves. This study provides new data on AMR in beef cow-calf operations in Florida, systematically comparing small, medium, and large beef cow-calf operations. Most studies have reported AMR at either the animal or isolate level, but we examined both, providing a more detailed view of resistance patterns. Unlike previous research, this study evaluated resistance patterns at both the animal and isolate levels and compared cows versus calves, revealing age- and farm size-related differences in AMR.

Variations in the frequency of resistance reported in different studies may be attributed to several factors. Primarily, studies often use different AST methods and interpretive breakpoints, which can lead to inconsistent classification of resistant isolates. Additionally, reporting formats vary, with some studies presenting data at the isolate level and others reporting data at the animal or farm level. Secondly, while antibiotic usage is frequently cited as a major driver of AMR, not all studies have found a consistent association between antimicrobial use and resistance patterns. This finding suggests that other factors may also play a significant role. In our study, we specifically found differences in resistance patterns based on farm size, suggesting that operation size may be an important factor influencing AMR in beef cow-calf systems. Although we did not assess other factors, previous research has considered variables, such as animal age, season, geographic location, hygiene, and environmental exposure. These findings collectively point to the complexity of AMR dynamics, highlighting the need for continued surveillance and standardized methodologies to improve comparability between studies.

This study has several limitations that should be acknowledged. First, we used a convenience sample of nine beef cow-calf operations, which may have introduced selection bias. Farm participation was voluntary and depended on the owners' willingness and existing relationships with university extension services. However, it includes operations from all three major operation-size categories (small, medium, and large) that are broadly representative of the beef cow-calf industry structure in the state. Second, due to the logistical challenges inherent to working with large-scale beef operations, it was not feasible to conduct random sampling at the animal level. This limited the generalizability of our findings. Third, detailed and documented antimicrobial use (AMU) records were unavailable. Because AMU is a key driver influencing the development and spread of AMR, the lack of farm-level treatment histories limits our ability to directly associate resistance patterns with antimicrobial exposure. In most cases, treatment information is based on owners' recollections, introducing the possibility of recall bias. Despite these limitations, the study's internal validity was maintained, as sample collection was standardized, laboratory methods were validated, and statistical analyses were rigorous, supporting the reliability of the reported AMR patterns.

## 5. Conclusions

This study provides new data on AMR in beef cow-calf operations in Florida, a sector where limited research exists. Across the nine participating farms (three small, three medium, and three large), resistance to at least one antibiotic and MAR was more frequent in large operations than in medium or small operations. At both the animal and isolate levels, the resistance patterns followed this trend. Resistance profiles also differed between cows and calves, suggesting that age may influence the occurrence and distribution of AMR within operations. These findings indicate that, within the nine studied beef cow-calf operations, farm size and animal age play important roles in AMR dynamics in beef cow-calf systems. We recommend that future research be expanded to include a larger number of beef cow-calf operations and incorporate additional factors, such as detailed antimicrobial use records, environmental conditions, and biosecurity measures, to better understand the drivers of AMR in beef cow-calf operations.

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**Data Availability Statement:** The raw data of this article will be made available by the corresponding author upon request.

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