# ANTIMICROBIAL RESISTANCE OF GENERIC *ESCHERICHIA COLI* IN CATTLE FECES AS IMPACTED BY DIETARY SUPPLEMENTATION WITH *LACTOBACILLUS SALIVARIUS* L28

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**Abstract – The purpose of this study was to determine the antimicrobial resistance (AMR) of Generic *Escherichia coli* isolated from fecal samples (n=72) and carcass swabs (n=215) of cattle fed three different diets with and without sub-therapeutic antibiotic supplementation and direct-fed microbial (L28) supplementation. *E. coli* was isolated using traditional culture methods, and AMR testing was performed using the NARMS protocol. *E. coli* was detected in 100% of the fecal samples, and MDR was detected in 16.6% of the CONTROL group (with tylosin), and 4% in both the BASE (no tylosin or DFM) and MONPRO groups (with DFM). *E. coli* was detected in 30% of carcass swabs, with the greatest MDR found in the cooler swabs (33.3%). Supplementation with DFM L28 resulted in similar AMR patterns as observed in cattle fed diets with no sub-therapeutic antibiotics indicating it could potentially reduce AMR in *E. coli* in cattle feces.**

**Key Words – DFM, feedlot cattle, Multi-drug resistance, *E. coli***

## I. INTRODUCTION

Antibiotics have been used for decades to help improve animal and human health, which could be a contributing factor to the increase and emergence of antimicrobial resistance globally [1]. Specifically, for production animals, antibiotics are largely used as antimicrobial growth promoters (AGP's), however with recent changes to the veterinary feed directive this sub-therapeutic use has been limited in the U.S. [2]. Thus, alternatives need to be investigated to help reduce the antimicrobial resistant (AMR) found in pathogens shed from the feces, while also maintaining cattle performance. Direct-fed microbials (DFM) have been shown to be an effective alternative to improve cattle daily gain and feed efficiency, increase milk production in dairy cows [3], while also reducing the shedding of pathogenic bacteria, such as *Escherichia coli* O157:H7 in the feces [4]. However, the impact of DFM on the presence of AMR bacteria has not been extensively studied.

## II. MATERIALS AND METHODS

Three dietary treatments based on high concentrate diets were fed to finish cattle for harvest: CONTROL (tylosin (88 mg/animal/day of diet DM) and monensin (330 mg/animal/day of diet DM)), MONPRO (a newly isolated DFM, *L. salivarius* L28, at a feeding rate of $10^6$ cfu/head/day, with monensin, but no tylosin), and BASE (no DFM, tylosin or monensin). Fecal samples (n=72), 1 per animal, were collected by rectal grabs before harvest. Samples were weighed, and enriched with phosphate buffer saline (PBS), and plated onto MacConkey agar for isolation of generic *E. coli*. Carcass swabs (n=215) were collected from the 72 carcasses at three different locations during harvest: pre–evisceration (n=72), post-evisceration (n=71), and from the cooler (n=72), using a sterile pre-hydrated swab containing buffered peptone water (BPW). Aliquots from each swab were transferred to MacConkey agar for isolation of generic *E. coli*.

From each MacConkey agar plate, 1 phenotypical colony was streaked onto 5% sheep blood agar plates (BAP) and subjected to antibiotic susceptibility testing. Antimicrobial resistance was analyzed using the micro-broth dilution (Sensititre™) susceptibility minimum inhibitory concentration (MIC) plates, following the National Antimicrobial Resistance Monitoring System (NARMS) protocol. Resistance and susceptible breakpoints were determined from the Clinical and Laboratory Standard Institute (CLSI).

## III. RESULTS AND DISCUSSION

*E. coli* was isolated from 100% (n=72) of fecal samples collected. Twenty-nine percent (n=7) of isolates in the CONTROL group were resistant to at least one antibiotic, and 16.7% (n=4) of isolates were multi-drug resistant

(MDR), resistance to 3 or more drugs. Isolates from the MONPRO treatment group showed 25% (n=6) resistance to one drug and 4.2% (n=1) were MDR. Twenty-nine percent (n=7) of isolates in the BASE treatment group had resistance to one drug and 4.2% of isolates were MDR. Specific antibiotics with resistance are listed in Table 1 for both fecal samples and carcass swabs.

From the 215 carcass swabs analyzed, *E. coli* was isolated from 30% (n=65) of samples, and from those 12.5% (n=9) were from pre-evisceration, 40.8% (n=29) from post-evisceration, and presence was detected in 37.5% (n=27) of samples from the cooler. Sixty-six percent (n=6) of pre-evisceration isolates were resistant to at least one antibiotic, and 11% (n=1) were MDR. Resistance was detected in 72.7% (n=8) of the post-evisceration isolates, and 27.3% (n=3) were MDR. Ninety-two percent (n=25) of isolates from the cooler were resistant to one antibiotic and 33% (n=9) were MDR.

Table 1. Percent of Generic *E. coli* isolates with antibiotic resistance for fecal samples and carcass swabs by specific antibiotic[1]. [1]STREPT=Streptomycin, TETRA=Tetracycline, CHLORA=Chloramphenicol, AMPICI=Ampicillin, AMOCLA=Amoxicillin, CEFOXI=Cefoxitin, CEFTIF=Cefriaxone, CEFTRI=Cefiofur, NALAC=Nalidixic Acid, TRISUL=Trimethoprim. Within the table n representing the number of isolates analyzed for the specific treatment group or carcass swab location. [2]Fecal samples by treatment. [3]Carcass swabs by location.

| | N | **STREPT** | **TETRA** | **CHLORA** | **AMPICI** | **AMOCLA** | **CEFOXI** | **CEFTIF** | **CEFTRI** | **NALAC** | **TRISUL** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CONTROL[2] | 24 | 8.3 | 29.2 | 4.2 | - | - | - | - | - | - | - |
| BASE[2] | 24 | 20.8 | 29.2 | 16.7 | 4.2 | - | - | - | - | - | - |
| MONPRO[2] | 24 | 8.3 | 25.0 | 4.2 | - | - | - | - | - | - | - |
| Pre-Evis[3] | 9 | 33.3 | 11.1 | - | 11.1 | 11.1 | 33.3 | - | - | - | - |
| Post-Evis[3] | 11 | 18.2 | 27.3 | 18.2 | 27.3 | 9.1 | 27.3 | 9.1 | 9.1 | 9.1 | - |
| Cooler[3] | 27 | 14.8 | 25.9 | 7.4 | 70.4 | 22.2 | 37.0 | 29.6 | 3.7 | 3.7 | 3.7 |

## IV. CONCLUSION

From fecal samples the most MDR was observed in the CONTROL fed group which had both tylosin and monensin supplementation. MDR in the isolates collected from the cattle fed the BASE and MONPRO diets were the same, with only 1 isolate having MDR from each respective group. The MONPRO diet had a greater number of isolates (n=18) susceptible to all antibiotics tested than other treatment groups (n=17). The supplementation of L28 instead of tylosin resulted in fewer MDR *E. coli.*

The presence of *E. coli* on the carcass swabs increased as the carcasses entered the cooler. *E. coli* is an indicator organism for fecal contamination, allowing us to conclude that there could be contamination happening within the production chain and may not be directly associated with the dietary treatments. Furthermore, the cooler swabs are also of more concern as they had greater MDR than at the other locations during harvest. Resulting in a greater risk of MDR *E. coli* entering the food supply system. This data reinforces the need to address the load of AMR *E. coli* entering and leaving the commercial abattoir.

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