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Antimicrobial resistance of *Escherichia coli* from dairy farms participating in an antimicrobial stewardship educational program for farm employees

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ABSTRACT

Antimicrobial use in food-producing animals is under increasing scrutiny due to the potential impact on the selection of antimicrobial-resistant bacteria that may be transmitted to humans by direct contact, with the food chain, or the environment. Novel data monitoring commensal *E. coli* from dairy farms is essential for understanding antimicrobial resistance (AMR) patterns and their association with herd health management practices. The objectives of this study were to: 1) compare the prevalence of antimicrobial resistance in the *E. coli* isolates from the hospital, fresh, and mid-lactation pens from 18 conventional dairy farms participating in an educational training program in antimicrobial stewardship practices in California and Ohio, and 2) to characterize the prevalence of antimicrobial resistance of commensal *E. coli* isolated from pooled fecal pat samples before and 3 mo after participating in the educational training program. Pooled fecal pat samples were collected from the hospital pen, the fresh pen (1 to 5 DIM), and the mid-lactation pens (90 to 150 DIM) on conventional dairies in CA (n = 9) and OH (n = 9). Fecal samples were collected as part of a larger study using a quasi-experimental design that assigned farms to the training intervention group (TG; 9 per state) or the control group (CG; 3 per state). For the TG, farm worker(s) identified as having the task of diagnosis and treatment of adult cows on the farm participated in a training program on antimicrobial stewardship practices. Pooled fecal samples (n = 7) were collected at enrollment and 3 mo after completing the intervention on each of the participating farms (n = 18), followed by culture for *E. coli* isolation and antimicrobial sensitivity testing using the broth microdilution methodology. Logistic regression models were used to evaluate the association between *E. coli* antimicrobial resistance patterns with the training intervention and farm-level factors. No effect was observed in the prevalence of resistant isolates between the control and intervention farms after the training was delivered. Isolates from the hospital pens were 2.48 (95% CI: 1.06 – 6.22, $P = 0.03$) and 5.61 (95% CI: 1.94 – 16.91, $P < 0.001$) times, more likely to be resistant to streptomycin and chloramphenicol, respectively, than isolates from the mid-lactation pens. Our findings indicate there was a higher prevalence of AMR in *E. coli* associated with the hospital pen within the farm, while the training program for 3 mo did not affect the prevalence of AMR in *E. coli* on the farms participating in the program. Further research efforts should be conducted to identify factors driving AMR at the pen level, as well as approaches that could be used to reduce the risk of disseminating AMR from sick pens to animals being housed and to other pens on the farm.
Keywords: dairy cattle, antibiotic resistance, multidrug resistance, lactation, pen

INTRODUCTION

Antimicrobial resistance (AMR) is one of the most urgent public health challenges of our time (CDC, 2019). Antimicrobial use in food-producing animals is under increased scrutiny due to the potential impact on the selection of antimicrobial-resistant bacteria, which may later be transmitted to other animals and humans through direct contact (e.g., farm workers), the food chain, or through the environment (Chantziaras et al., 2014; Manaia, 2017).

Judicious use of antimicrobials on the farm, despite requiring veterinarian oversight and a veterinarian-client-patient relationship (VCPR), relies heavily upon dairy workers' skills to accurately detect and treat sick animals daily (Espadamala et al., 2016, 2018). Therefore, educational programs to improve farmworkers' knowledge and attitudes toward antimicrobial steward-

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ship on the farm are also critical in achieving judicious use of antimicrobials while maintaining animal health and welfare.

Current AMR is monitored and published annually through surveillance programs such as the US National Antimicrobial Resistance Monitoring System integrated report (NARMS, 2019a), which efforts are focused on post-harvest locations. Thus, there is a knowledge gap for on-farm AMR monitoring data and that could result in more effective efforts to reduce the selection and dissemination of AMR from cattle.

AMR surveillance requires a holistic approach to identify trends in resistance to selected antimicrobials. *E. coli* is a fecal commensal bacterium used as an indicator organism for antimicrobial resistance surveillance as well as a potential source of resistance genes (EFSA, 2008). Horizontal transfer of AMR genes from *E. coli* to pathogenic bacteria poses a risk to public health. Monitoring commensal *E. coli* from dairy farms is a crucial step for understanding AMR patterns and profiles. Resistance patterns are the description of the antibiotic resistance testing results for an isolate, while resistance profiles are the description of the resistance patterns for all isolates in an investigation (NARMS, 2019b). Detecting trends in phenotypic resistance relevant to public health and their relation to different herd health management practices could be used as an approach to evaluate on-farm antimicrobial stewardship program implementation.

Different factors influence the AMR patterns of *E. coli* such as age (Berge et al., 2005; Pereira et al., 2015; Cao et al., 2019), geographic location (Berge et al., 2010; Abdelfattah et al., 2021), production systems (Berge et al., 2005; Enne et al., 2008; Hailu et al., 2021), and previous antimicrobial treatments (Pereira et al., 2014, 2020; Duse et al., 2015), among other factors. Therefore, identifying risk factors associated with AMR on dairy farms can help identify areas on which to focus antimicrobial stewardship practices.

Our first objective was to compare the prevalence of antimicrobial resistance in the *E. coli* isolates from the hospital, fresh, and mid-lactation pens in 18 farms participating in an educational training program in antimicrobial stewardship practices. We hypothesized that farms participating in the training would have a significantly lower prevalence of AMR in *E. coli* in fecal pats compared with pre-training samples. Our second objective was to characterize the prevalence and patterns of antimicrobial resistance profiles on commensal *E. coli* isolated from the farms in California and Ohio before and after participating in the educational program. We hypothesized that the hospital pens would have a significantly higher prevalence of AMR in *E. coli* in fecal pats when compared with the fresh and mid-lactation pens.

METHODS

All procedures conducted were approved by The Ohio State University Institutional Review Board (*#2019B047*) and the study was conducted from August 2020 to March 2022.

Study design

Environmental pooled fecal pat samples were collected from the hospital pen (cows that have received antimicrobial treatment with milk withhold period), the fresh pen (1 to 5 d postpartum), and the mid-lactation pens (90 to 150 DIM), on conventional dairies in California (n = 9) and Ohio (n = 9). Fecal samples were collected as part of a larger study with a quasi-experimental design that assigned farms to the training intervention group (TG; 6 per state) or control group (CG; 3 per state). For the TG, farmworker(s) (n = 25) identified as having the task of diagnosis and treatment of adult cows on the farm participated in a 12-week training program on antimicrobial stewardship practices as described in Garzon et al., (2023). Briefly, 6 training modules were developed and delivered as interactive short videos with audio using a case-based teaching approach to cover the learning objectives for each module: antimicrobial resistance, treatment protocols, visual identification of sick animals, clinical mastitis, puerperal metritis, and lameness. All materials were available in Spanish and English. Pre- and post-training assessments were administered using an online training assessment tool to evaluate changes in knowledge and attitudes about antimicrobial stewardship practices.

Composite fecal samples (~200 g/sample) were collected from the floor of each pen by pooling feces from 10 fresh fecal pats using a 20 mL sterile sampling spoon. One composite sample was collected from the hospital pen, 3 composite fecal samples were collected from one fresh pen, and 3 composite samples from one mid-lactation pen, for a total of 7 composite samples in each farm. Each composite sample was placed into an 18 Oz Whirl-Pak bag (Nasco, Fort Atkinson, Wisconsin) and mixed by hand thoroughly. After collection, fecal samples were immediately placed on ice and transported to a laboratory for processing.

Samples were collected twice during the study: at enrollment (Time 1) and 3 mo after finishing the 12-week educational training program (Time 2). By the end of the study, 252 pooled fecal samples were collected for antimicrobial susceptibility testing.

Bacterial Isolation and Antimicrobial Susceptibility Testing

Within 24 h after collection, each pooled fecal sample was used to inoculate a single CHROMagar-*E. coli* selective plate (CHROMagar Microbiology, Paris, France) which was then incubated aerobically at 37°C for 24 h. Two individual isolates were selected and subcultured individually in 10 mL of sterile Luria-Bertani broth (Difco; Becton, Dickinson, and Company, Sparks, MD, USA) at 37°C for 24 h. The broth culture (0.5 mL) was mixed with 50% sterile glycerol/50% sterile water solution (0.5 mL) before storage at −80°C.

A total of 504 *E. coli* isolates were evaluated for antimicrobial susceptibility using a microbroth dilution method following the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022). The Sensititer NARMS Gram-Negative Plate (CMV3AGNF, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used for testing susceptibility to the following 14 antimicrobial drugs: aminoglycosides (gentamicin and streptomycin), β–lactam combination (amoxicillin/clavulanic acid), cephems (cefoxitin, ceftriaxone, and ceftiofur), folate pathway antagonists (trimethoprim/sulfamethoxazole and sulfisoxazole), macrolides (azithromycin), penicillins (ampicillin), phenicols (chloramphenicol), quinolones (ciprofloxacin and nalidixic acid) and tetracyclines (tetracycline). Sensititer plates were read manually, and minimum inhibitory concentrations were interpreted as susceptible, intermediate, and resistant using current CLSI breakpoints (CLSI, 2022) (Supplemental Table 1)(Garzon et al., 2023b). Isolates that grew in all dilutions of an antimicrobial assessed (sulfisoxazole) were classified as "growth in all dilutions" (GAD) because their MIC was higher than the highest dilution tested in our study, hence the actual MIC value is unknown. As CLSI interpretive criteria for streptomycin, azithromycin, and ceftiofur for *E. coli* are lacking, the National Antimicrobial Resistance Monitoring System interpretive criteria were used instead (NARMS, 2019). A reference strain of *E. coli* (ATCC 25922) was used as quality control and run weekly. Isolates were classified as multidrug-resistant (MDR) when they were resistant to at least one drug in 3 or more antimicrobial classes.

Statistical analysis.

Statistical analyses were conducted using SAS (SAS Institute Inc., Cary, NC; version 8.3.0). The proportions of resistant isolates and associated 95% Clopper–Pearson confidence interval (CI) were descriptively reported for each antimicrobial drug across the state (California and Ohio) and pen (hospital, fresh and mid-lactation) within each treatment group (treatment and control) and sampling point (enrollment and end of intervention). A kappa statistic was conducted to evaluate the degree of agreement between the 2 isolates from the same pooled fecal sample for the classification of the isolate as MDR.

Logistic regression models in SAS using PROC GLIMMIX logit function were used to evaluate the association between *E. coli* antimicrobial resistance profile and the specific pen where samples were collected (hospital pen, fresh pen, mid-lactation pen) on each farm, to assess the effect of the pen on the AMR of the isolates. All models included the farm as a random effect, and samples were nested within the farm. To control for confounders, variables that changed the coefficients of remaining variables greater than 30% were checked. The Akaike information criterion (AIC) was used for model selection and to assure a more parsimonious model was selected. All p-values were adjusted for multiple testing using a Bonferroni correction.

Logistic regression models were also used to evaluate the effect of the educational intervention on the *E. coli* antimicrobial resistance profile. A univariate analysis was conducted for each of the explanatory variables (pen, state, time points, treatment group) using the Chi-squared test (χ^2 test), and variables with a $P < 0.30$ were offered to the logistic regression model using a backward stepwise elimination process. A model was generated for each antimicrobial evaluated, excluding azithromycin and sulfisoxazole, given that isolates were fully susceptible to both antimicrobials. A model using a multidrug resistance (MDR) binomial variable as an outcome was also evaluated. The MICs breakpoints were used to categorize the outcome as a binomial variable classifying an isolate as resistant or susceptible. Isolates with an intermediate classification according to their MIC breakpoint were grouped as susceptible in the binomial variable. All models included the farm as a random effect and samples were nested within the farm. To control for confounders, variables that changed the coefficients of remaining variables greater than 30% were checked. The Akaike information criterion (AIC) was used for model selection and to assure a more parsimonious model was selected. All p-values were adjusted for multiple testing using a Bonferroni correction.

RESULTS

A total of 252 pooled fecal samples were collected for the study. The information on the 18 enrolled study farms is summarized in Table 1. All farms used a free-stall housing management system. The outline of the number of samples from the *E. coli* isolates obtained by the time point, state, and pen is presented in Figure 1.

The distribution of the minimum inhibitory concentration (MIC) and resistance for *E. coli* (n = 504) by the individual antimicrobial drug is shown in Table 2. All isolates were susceptible to azithromycin. For sulfisoxazole, the MIC for all isolates was greater than the highest concentration evaluated which could not be accurately classified and was excluded from the analysis. The 3 most common antimicrobial drugs to which isolates were resistant were tetracycline, followed by streptomycin, and ampicillin (Table 2). The top 15 most common AMR patterns are shown in Table 3 and the complete list of AMR profiles is shown in Supplemental Table 2 (Garzon et al., 2023b). Multidrug resistance (MDR) was defined as resistance to 3 or more drug classes and was found in 15.2% (n = 77/504) of the isolates, with a diversity of 57 distinct AMR patterns (Supplemental Table 3) (Garzon et al., 2023b). There was a moderate agreement between the 2 isolates from the same pooled fecal sample for the classification of the isolate as MDR (kappa: 0.37, 95% CI: 0.26 to 0.47). The commonly observed MDR patterns were streptomycin-ceftriaxone-tetracycline (n = 5), streptomycin-chloramphenicol-tetracycline (n = 5), and streptomycin-ampicillin-chloramphenicol-tetracycline (n = 4). MDR prevalence was 19, 33, and 25% for the hospital, fresh, and mid-lactation pens, respectively. At the farm level, MDR prevalence varied between 3.5 to 39.2%, and MDR isolates were retrieved from 14 of the 18 participating farms. The prevalence of resistant isolates to any of the antimicrobial drugs evaluated between farms was not statistically significant. The percentage of resistance in *E. coli* isolates in each of the participating farms is shown in Supplemental Figure 1 (Garzon et al., 2023b).

The percentage of resistant isolates for each antimicrobial drug or classified as MDR did not statistically differ between the control and intervention farms after the training was delivered (Figure 2). The complete results from the logistic regression are shown in Supplemental Tables 4 and 5 (Garzon et al., 2023b).

The percentage of resistant isolates for each antimicrobial drug did not statistically differ between California and Ohio after the training was delivered (Supplemental Figure 2) (Garzon et al., 2023b). The percentage of resistant isolates for each antimicrobial drug from the hospital, fresh, and mid-lactation pens did not statistically differ between the control and intervention farms after the training was delivered (Supplemental Figure 3) (Garzon et al., 2023b). The complete results from the logistic regressions are shown in Supplemental Tables 6 and 7 (Garzon et al., 2023b), respectively.

For the logistic regression evaluating the association between the AMR prevalence within the pen, independent of the intervention effect, there were significant differences between the hospital pen compared with the mid-lactation and the fresh pen for streptomycin and chloramphenicol (Figure 3A). Isolates from the hospital pen were 2.48 (95% CI: 1.06 – 6.22, $P = 0.03$) and 5.61 (95% CI: 1.94 – 16.91, $P < 0.001$) times, more likely

Table 1. Descriptive characteristics of nine California and nine Ohio dairy farms enrolled in a study to determine the antimicrobial resistance profiles of *E. coli* isolated from pooled fecal samples of dairy cows

Farm	State	Herd size[1]	Breed	RHA, Kg/cow[2]
1	CA	1,000	Holstein and Jersey	NR
2	CA	1,380	Holstein and Jersey	9,825
3	CA	1,343	Holstein and Jersey	9,131
4	CA	675	Holstein	10,024
5	CA	1,360	Jersey	9,370
6	CA	3,400	Holstein	13,926
7	CA	6,100	Holstein	NR
8	CA	6,000	Holstein	12,525
9	CA	2,200	Holstein and Jersey	8,893
10	OH	1,200	Holstein	9,375
11	OH	630	Holstein	11,804
12	OH	900	Holstein	9,785
13	OH	4,900	Holstein	12,657
14	OH	1,150	Holstein	13,094
15	OH	1,060	Holstein	13,882
16	OH	4,000	Holstein	NR
17	OH	1,000	Holstein	12,999
18	OH	2,000	Holstein	10,330

[1]Mean milking herd size.

[2]Rolling herd average, mean milk produced per milking cow in the herd during the previous year.

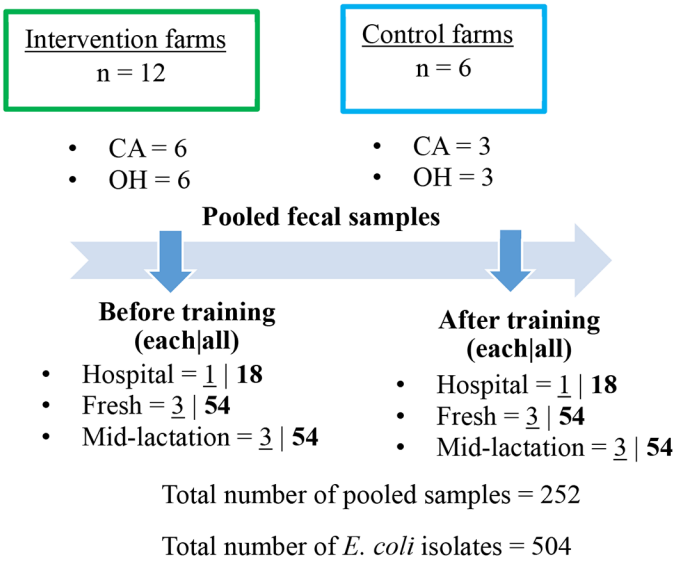

Figure 1. The number of enrolled farms, the pooled fecal samples collected, and *E. coli* isolates by farm and sampling point. Numbers of pooled fecal samples collected by pen in each farm (underlined), and in total (bold) for the 18 farms before and after the intervention.

to be resistant to streptomycin and chloramphenicol, respectively, than isolates from the mid-lactation pen. Similarly, isolates from the hospital pen were 5.11 times (95% CI: 1.8 – 14.4, $P < 0.0001$) more likely to be resistant to chloramphenicol than isolates from the fresh pen (Table 4).

For the logistic regression evaluating the association between the MDR prevalence within the pen, there was a significant difference between the hospital compared with the mid-lactation pen (Figure 3B). Isolates from the hospital pen were 3.1 (95% CI: 1.14 – 8.43, $P = 0.02$) times more likely to be multidrug resistant than isolates from the mid-lactation pen.

DISCUSSION

There are increasing concerns with antimicrobial use in food-producing animals as they potentially can favor the selection of antimicrobial-resistant bacteria. Implementing antimicrobial use stewardship programs on farms can be a mitigation strategy. Our study investigated the efficacy of implementing antimicrobial stewardship educational training programs in California and Ohio dairy farms to reduce AMR in fecal *E. coli*. We also explored differences in AMR between different lactating pens (hospital, fresh, and mid-lactation). Changes in AMR in fecal *E. coli* from fresh, mid-lactation and hospital pens of dairy farms were not observed after a 3-mo training program for dairy workers on animal health disorders identification and treatment. A recent systematic review revealed that strategies aimed at enhancing antimicrobial stewardship practices resulted in inconsistent results when the expected outcome is the reduction in the prevalence or number of antimicrobial resistance genes (Nobrega et al., 2021). Furthermore, the reduction in AMR prevalence at the pen or farm level could take a longer time or may be influenced by other genetic, ecological, or metabolic factors (Bottery et al., 2020) such as AMR co-selection (Aarestrup, 2000), microbial collective resistance (Sorg et al., 2016) or the variation in the persistence of mobile genetic elements despite segregational loss (Carroll and Wong, 2018). However, active long-term antimicrobial resistance monitoring at the farm may be a valuable tool as part of the evaluation of antimicrobial stewardship programs.

The low prevalence of AMR for fecal *E. coli* in adult cattle observed in our study has also been reported by other authors in Pennsylvania, California, Canada, and Great Britain (Enne et al., 2008; Cao et al., 2019; Abdelfattah et al., 2021; Massé et al., 2021). A study evaluating the prevalence and resistance of *E. coli* from dairy animals in 80 Pennsylvania dairy herds, also found a lower prevalence of *E. coli* resistant to antimicrobials in composite fecal samples in adult dairy cattle than in calves (Cao et al., 2019). Differences in AMR prevalence may be due to selective pressure, given differences in management practices such as feeding waste milk or medicated milk to pre-weaned calves (Pereira et al., 2014; Maynou et al., 2017), and higher disease and treatment incidence in younger animals (Berge et al., 2005; Awosile et al., 2018; Springer et al., 2019). Future research evaluating the effect of training programs for disease identification in both adult cattle and calves, as well as the AMR profile of the dairy calves of the farms may provide a more complete understanding of the prevalence of antimicrobial resistance in bacteria on the farm and the management practices associated with the selection of the resistance.S

Our low AMR profile for tetracycline in fecal *E. coli* isolates agrees with prior findings from lactating dairy cows in California and Pennsylvania herds (~15%; Cao et al., 2019; Abdelfattah et al., 2021). Accordingly, the 2019 dairy cattle NARMS reported a 16.8% AMR prevalence of tetracycline (FDA, 2019). Similarly, in Canada, *E. coli* isolates from pooled adult dairy cattle reached a resistance to tetracycline of 15% (Massé et al., 2021). According to the NAHMS report, tetracyclines were used as the primary antimicrobial for reproductive disease in 13.3% and lameness in 11.4% of dairy operations in the US (USDA, 2018).

Following resistance to tetracycline, the most common drugs for which AMR was observed were ampicillin (11.1%), chloramphenicol (8.3%), and streptomycin (16.2%). However, our findings were higher than the 2019 NARMS report, in which the resistance levels were 4.7, 4.0, and 9.7%, respectively. Chloramphenicol was never approved for the treatment of food-producing animals in the US, and it has been banned for extra-label use since the 1980s (Gilmore, 1986). Resistance to this antimicrobial drug may come from cross-resistance with florfenicol, currently indicated for the treatment of respiratory diseases in dairy cattle younger than 20 mo of age (White et al., 2000; Schwarz et al., 2004). Cross-resistance or indirect resistance between chloramphenicol and tetracycline (Okamoto and Mizuno, 1964; Cohen et al., 1989) or β-lactams has been described before, but the specific mechanisms is not completely understood (Nicoloff H. and Andersson, 2015).

Among cephalosporins, ceftiofur had the lowest proportion of resistance (1.9%), while the resistance to cefoxitin (6.1%) and ceftriaxone (10.7%) was comparatively higher. Ceftiofur is a third-generation cephalosporin and one of the most common antimicrobials used in US dairies for treating lactating dairy cattle (USDA, 2018). Currently, only ceftiofur and cephapirin are labeled to use in cattle (FDA, 2022). Ceftriaxone is also a third-generation cephalosporin, as is ceftiofur.

Table 2. Distribution of minimum inhibitory concentration (MIC) and resistance for *E. coli* (n = 504) by individual antimicrobial drug for the CMV3AGNF panel. Highlighted areas in green correspond to susceptible, yellow correspond to intermediate, and red corresponds to resistant classification (CLSI, 2022)

		% Distribution of MICs (μg/ml)												
Antimicrobial	R[1%]	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
Amoxicillin/clavulanic acid	6.5							4.96	23.41	53.57	8.73	2.78	6.55			
Ampicillin	11.1							12.1	54.96	14.88	4.17	2.78	11.11			
Azithromycin[2]	0.00				0.6			4.96	45.23	41.07	7.94	0.2				
Cefoxitin	6.1						0.2	0.2	3.77	35.91	43.65	10.12	6.15			
Ceftriaxone	10.7						84.13	1.39	1.59	2.18	2.58	1.98	2.38	1.39	2.38	
Ceftiofur[3]	1.9				2.18	21.03	60.71	7.54	3.17	3.37	1.98					
Chloramphenicol	8.3								2.78	42.66	40.87	5.36	8.33			
Ciprofloxacin	4.5	82.94	2.78	2.18	1.79	3.37	2.38	1.39	1.98	1.19						
Gentamicin	3.3					3.57	38.89	42.46	7.94	2.78	0.99	3.37				
Nalidixic Acid	2.1						0.2	10.52	71.83	10.12	3.17	1.98	2.18			
Streptomycin[2]	16.2								1.19	15.48	53.77	13.29	6.55	9.72		
Sulfisoxazole	0.00											17.46	5.16	8.53	0.99	67.86
Tetracycline	18.2									80.75	0.99	1.98	16.28			
Trimethoprim/sulfamethoxazole	4.1				87.3	1.19	2.18	2.98	2.18	4.17						

[1]Percentage of isolates classified as resistant to the individual antimicrobial drug.
[2]CLSI breakpoints are not established; interpretive standards used are NARMS-established breakpoints for resistance monitoring.
[3]NARMS, 2019.

Resistance against ceftriaxone represents a concern given its importance in the treatment of serious gram-negative bacterial infections infection in humans, and its close relationship with ceftiofur (Sato et al., 2014; Taylor et al., 2021). For ceftiofur, previous studies have found similarly low levels of resistance in adult cattle (Cao et al., 2019; Massé et al., 2021), while higher levels of resistance have been observed in younger animals (Springer et al., 2019). Factors such as age (Duse et al., 2015; Cao et al., 2019; Hordijk et al., 2019), and management practices (Pereira et al., 2014; Maynou et al., 2017; Awosile et al., 2018), influence the AMR profiles, which highlight the importance of understanding and considering those differences where planning effective antimicrobial stewardship practices on the farm. Differences in resistance between ceftiofur and ceftriaxone and cefoxitin may be related to the breakpoints used for defining resistance. For both ceftriaxone and cefoxitin, there are current MIC breakpoints available developed by the CLSI, while there are not for ceftiofur. Verification and validation of antimicrobial susceptibility methods and breakpoints for ceftiofur may be warranted to promote stewardship practices and treatment decisions.

All isolates from our study were susceptible to azithromycin. *Enterobacteriaceae* are reported to be intrinsically resistant to macrolides with exception of azithromycin (CLSI, 2022). Our results agree with previous results where isolates from lactating dairy cows were highly susceptible to azithromycin (97.8%, Tyson et al., 2015; 98.8%, Abdelfattah et al., 2021). This high susceptibility to azithromycin can be explained by a higher intracellular uptake compared with other macrolides, attributed to its cationic properties (Farmer et al., 1992; Gomes et al., 2017).

The 2 most common MDR profiles found in our study were streptomycin-ceftriaxone-tetracycline and streptomycin-chloramphenicol-tetracycline. Isolates from the hospital pen had significantly higher odds of being classified as MDR when compared with isolates from the mid-lactation pens. The prevalence of multidrug-resistant bacteria is of special concern given the implicated risk of limiting the options for treating an infection in both humans and animals (Brichta-Harhay et al., 2011; Doyle, 2015; Walther et al., 2017). This finding highlights the importance of continuing surveillance of resistance patterns and a better understanding of the resistance in the dairy, to monitor and control the dissemination and spread of drug-resistant bacteria to the environment, other animals, and humans working in close contact with the animals.

Isolates from the hospital pen had significantly higher odds of resistance to streptomycin and chloramphenicol when compared with isolates collected from

the mid-lactation pen. To our knowledge, this is the first study comparing the resistance profile of *E. coli* between different pens based on disease and lactation stages within the farm. Previous research has investigated differences in resistance patterns between lactating and dry cows (Cao et al., 2019) and the variation in a cohort study conducted in California following cows from the close-up period up to 120 DIM (Abdelfattah et al., 2021). Differences found between the hospital and mid-lactation pen in our study could be due to the higher antimicrobial use and the consequent increase in antimicrobial resistance bacteria in the hospital pen. This finding highlights the importance of promoting the judicious use of antimicrobials, by improving disease diagnosis and treatment of cattle to avoid the unnecessary use of antimicrobials. This also highlights the need for further research to investigate the persistence over time of resistant bacteria after antimicrobial treatment and before cows leave the hospital pen to mitigate the risk of dissemination of resistance on the farm (Singer et al., 2008; Taylor et al., 2019).

This study evaluated *E. coli* AMR patterns from large conventional dairy farms in California and Ohio, before and after an educational program was conducted, which precludes us from generalizing our findings to other management practices. Further studies including small and medium size dairy farms, different management practices, and other states will generate results that provide a more comprehensive AMR panorama on the dairy industry. Minimum inhibitory concentration cutoffs to determine antimicrobial susceptibility are based on clinical treatment outcomes and may not be appropriate for environmental monitoring. Our study also only evaluated the change in resistance during a 3-mo period, which may have precluded us from finding any change in the resistance profile of the enrolled farms. Active long-term antimicrobial resistance monitoring at the farm may be a valuable tool as part of the evaluation of antimicrobial stewardship programs. Further studies evaluating the genetic elements present in the bacteria would provide a further understanding of the ecology and evolution of antimicrobial resistance in the bacteria communities, which provide information better understanding of the AMR dynamics at a farm level.

Table 3. Top 15 most common antimicrobial resistance patterns for *E. coli* isolated from dairy farms participating in an antimicrobial stewardship educational training program for farm employees (n = 414/504)

AMR profile	Number of isolates	Percent
pansusceptible	312	61.9
Tet	20	10.4
Str	14	7.2
StrTet	10	5.2
Axo	8	4.1
Amp	6	3.1
Chl	6	3.1
Fox	6	3.1
AxoTet	5	2.6
StrAxoTet	5	2.6
StrChlTet	5	2.6
Sxt	5	2.6
Cip	4	2.0
StrAmp	4	2.0
StrAmpChlTet	4	2.0

* Fox: cefoxitin, Gen: gentamicin, Str: streptomycin, Axo: ceftriaxone, Xnl: ceftiofur, Sxt: trimethoprim/sulfamethoxazole, Fis: sulfisoxazole, Azi: azithromycin, Aug: amoxicillin/clavulanic acid 2:1 ratio, Amp: ampicillin, Chl: chloramphenicol, Cip: ciprofloxacin, Nal: nalidixic acid, Tet: tetracycline.

CONCLUSION

Antimicrobial resistance in *E. coli* isolates on dairy cattle pooled fecal samples from the hospital, fresh and mid-lactation pens on 9 CA and 9 OH farms was similar to previous reports. We identified tetracycline, streptomycin, and ampicillin as the 3 most common antimicrobial drugs that isolates were resistant to. Isolates from the hospital pen had greater odds of resistance to streptomycin and chloramphenicol, as well as being multidrug resistant compared with the mid-lactation and fresh cow pens. We were not able to identify a change in antimicrobial resistance related to our educational program intervention aiming to improve farmworkers' knowledge and attitude toward antimicrobial stewardship. Our findings support the need for further research to better understand the long-term effects of antimicrobial stewardship farm practices driving AMR in lactating cattle, especially in the pens housing the sick animals.

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Conflict of Interest The authors declare that the research was conducted without any commercial or finan-

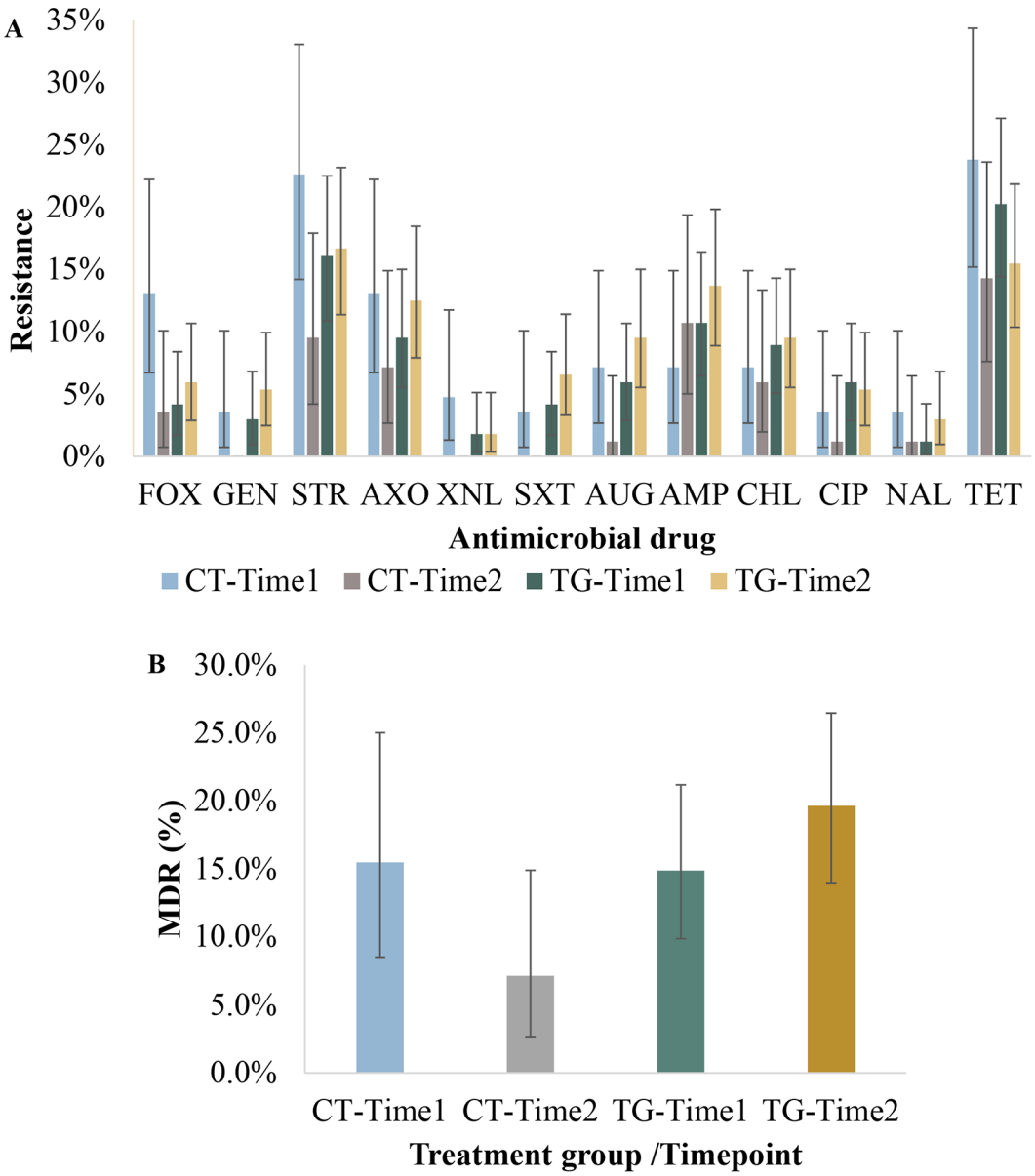

Figure 2. The proportion of resistant *E. coli* isolates from pooled fecal samples from control (CT) and intervention (TG) groups before (Time 1) and after (Time 2) participating in an educational program in antimicrobial stewardship **A)** to each tested antimicrobial and **B)** categorized as multidrug resistant. Error bars represent 95% CI.

cial relationships that could be construed as a potential conflict of interest.

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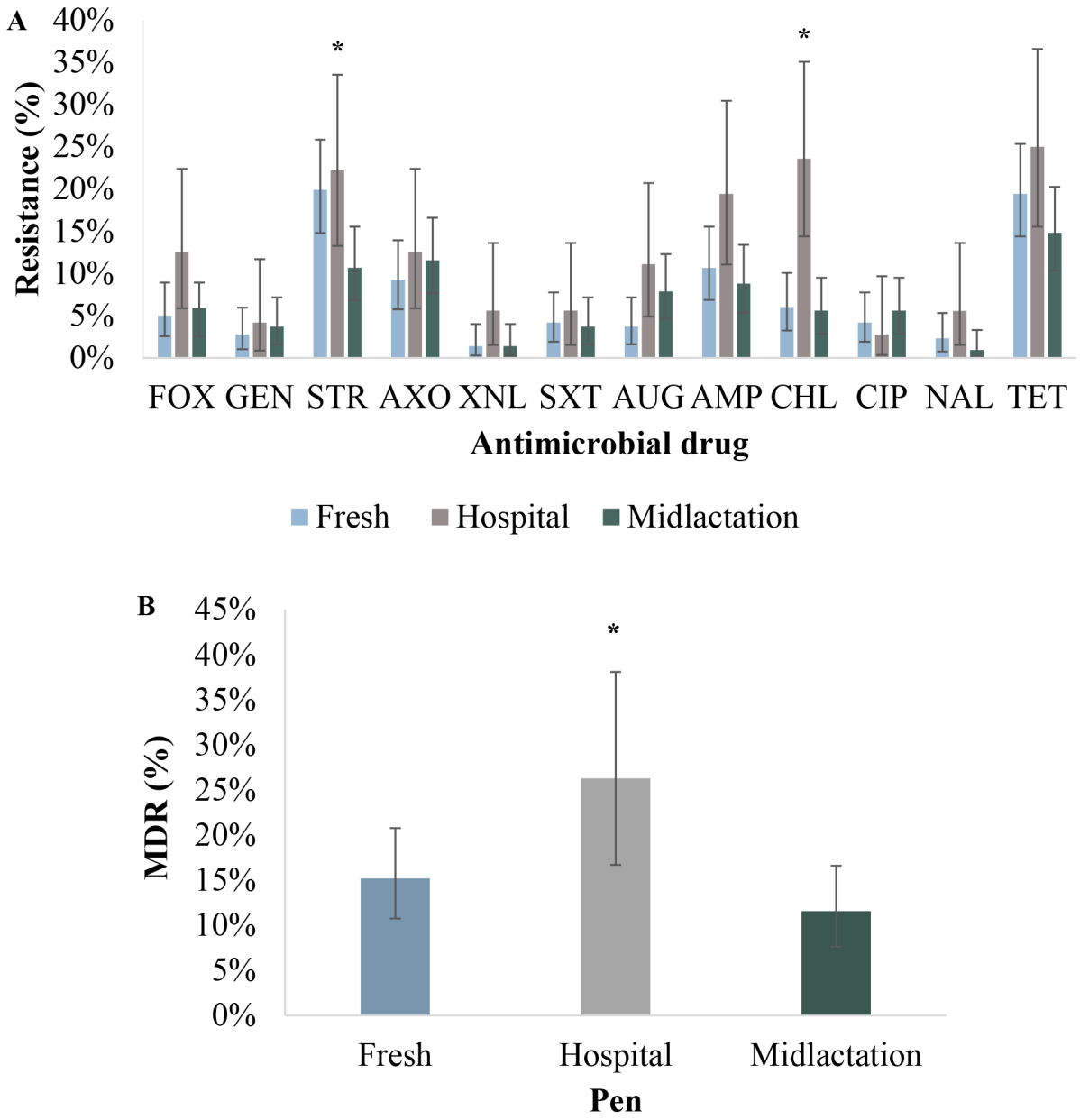

Figure 3. The proportion of resistant *E. coli* isolates from pooled fecal samples from the fresh cows' pen, the hospital pen, and the mid-lactation pen of dairy farms participating in an educational training program in antimicrobial stewardship before starting the intervention to **A)** each tested antimicrobial, and **B)** categorized as multidrug resistant. Asterisk represents a statistically significant difference at the logistic regression analysis ($P < 0.05$). Error bars represent 95% CI.

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Table 4. Summary of the logistic regression model evaluating the effect of the pen on the odds ratio of AMR prevalence in *E. coli* isolates

			OR 95% CI		
Antimicrobial	Variable	OR	Lower	Upper	p-value
STR					0.02
	Fresh vs Hospital	0.86	0.37	2.03	1.00
	Fresh vs Midlactation	2.14	0.99	4.34	0.05
	Hospital vs Midlactation	2.48	1.06	6.22	0.03
FOX					0.07
	Fresh vs Hospital	0.37	0.12	1.17	0.12
	Fresh vs Midlactation	1.00	0.35	2.88	1.00
	Hospital vs Midlactation	2.68	0.86	8.39	0.12
AUG					0.06
	Fresh vs Hospital	0.30	0.08	1.08	0.07
	Fresh vs Midlactation	0.45	0.15	1.30	0.21
	Hospital vs Midlactation	1.48	0.49	4.48	1.00
CHL					<.0001
	Hospital vs Fresh	5.11	1.8	14.8	<.0001
	Fresh vs Midlactation	1.10	0.39	3.09	1.00
	Hospital vs Midlactation	5.61	1.94	16.19	<.0001

* STR: streptomycin, FOX: cefoxitin, AUG: amoxicillin/clavulanic acid, CHL: chloramphenicol.

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