HHS Public Access

Author manuscript

J Food Prot. Author manuscript; available in PMC 2021 December 03.

Published in final edited form as:

J Food Prot. 2016 March ; 79(3): 484–487. doi:10.4315/0362-028X.JFP-15-321.

Antimicrobial Resistance Profiles in Escherichia coli O157 Isolates from Northern Colorado Dairies

CRAIG S. McCONNEL[1], **CHLOE M. STENKAMP-STRAHM**[1], **SANGEETA RAO**[1], **LYNDSEY M. LINKE**[1], **ROBERTA J. MAGNUSON**[1], **DOREENE R. HYATT**[2]

[1]Department of Clinical Sciences, Colorado Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, Colorado 80523, USA

[2]Department of Microbiology, Immunology and Pathology, Colorado Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, Colorado 80523, USA

Abstract

Escherichia coli O157 (*Ec*O157) infections can lead to serious disease and death in humans. Although the ecology of *Ec*O157 is complex, ruminant animals serve as an important reservoir for human infection. Dairy cattle are unique because they may be a source of contamination for milk, meat, and manure-fertilized crops. Foodborne dairy pathogens such as *Ec*O157 are of primary importance to public health. Antimicrobial resistance (AMR) is a complex phenomenon that complicates the treatment of serious bacterial infections and is of increasing concern. In the face of recommended use restrictions for antimicrobial agents in livestock operations, current AMR patterns in known foodborne pathogens should be documented. The objective of this study was to document AMR patterns in *Ec*O157 isolates from dairies in northern Colorado using antimicrobial agents commonly found on dairies and representative of medically important antimicrobial drug classes. Seventy-five *Ec*O157 isolates were recovered from three dairies. Six isolates were resistant to at least 1 of the 10 tested antimicrobial agents: four were resistant to streptomycin, sulfisoxazole, and tetracycline; one was resistant to streptomycin and tetracycline; and one was resistant to only tetracycline. All resistant isolates were from a single dairy. Overall, a low prevalence (8%) of AMR was observed among the 75 *Ec*O157 isolates. No significant effects on AMR profiles due to virulence genes, parity, or previous antimicrobial treatments within the current lactation period were detected. The results of this study provide background information for future comparative studies investigating AMR trends. Future studies should include more participating farms and more samples and should control for potential confounding factors of AMR that may underlie individual farm variation.

Keywords

Antimicrobial resistance; Dairy; *Escherichia coli* O157

Escherichia coli O157 (*Ec*O157) infections can lead to serious disease and death in humans (22). *Ec*O157 pathogenicity is related to the presence of virulence genes (12), including stx_1 and stx_2, which encode Shiga toxins, and *eaeA*, which encodes the protein intimin. Shiga toxin–producing *Ec*O157 that are classified as enterohemorrhagic *E. coli* (EHEC) are associated with hemorrhagic colitis and hemolytic uremic syndrome (5, 11). *Ec*O157

without the capacity to produce Shiga toxins still may contain the virulence gene *eaeA* and are classified as atypical enteropathogenic *E. coli* (aEPEC) (7). aEPEC strains can adhere to epithelial cells leading to attaching and effacing lesions (3, 4).

Although the ecology of *Ec*O157 is complex, ruminants serve as an important reservoir for human infection (18). *Ec*O157 is a commensal in the gastrointestinal tract of cattle (20) and is transmitted to humans by ingestion of contaminated foodstuffs or water or through direct contact with infected cattle or other hosts (8, 25). Some dairy products provide *Ec*O157 with favorable conditions for growth, and dairy cattle are unique in that they may be a source of contamination for milk, meat, and manure-fertilized crops. Thus, foodborne dairy pathogens such as *Ec*O157 are of primary importance to public health (7).

Antimicrobial resistance (AMR) is a complex phenomenon that complicates the treatment of serious bacterial infections and is of increasing concern. Although antimicrobial agents generally are not recommended for treating *Ec*O157 infections (32), administration of antimicrobial agents early in the infection may prevent the progression of disease to hemolytic uremic syndrome (13). Although the role of early antimicrobial therapy in *Ec*O157 infections still is unclear, the emergence and dissemination of AMR among *Ec*O157 and other zoonotic foodborne pathogens has negative clinical implications (19).

Currently, limited data are available related to *Ec*O157 antimicrobial susceptibility on dairies (1, 9, 17). Many factors can affect the frequency of resistance determinants among bacterial populations, including exposure to antimicrobial drugs and environmental conditions that affect the fitness of certain strains of bacteria harboring resistance genes (1, 28). Bacteria use a number of mechanisms to resist the effects of antimicrobial agents. These mechanisms include modifying the antimicrobial agent, altering the agent's target, decreasing cell wall access to the target, and implementing an alternative metabolic pathway not affected by the antimicrobial agent (16). According to the U.S. Food and Drug Administration (FDA) judicious use guidance (29), the use of antimicrobial drugs that are medically important in human medicine should be limited in animals used for food. The current list of medically important antimicrobial drug classes includes aminoglycosides, lincosamides, macrolides, penicillins, streptogramins, sulfonamides, and tetracyclines.

To better judge the effect of the recommended antimicrobial use restrictions on livestock operations, current AMR patterns in known foodborne pathogens should be documented. Surveillance of AMR among zoonotic foodborne pathogens such as *Ec*O157 is warranted to protect public health (19), and surveillance programs have been implemented worldwide to better understand AMR trends (2, 6, 24). The objective of this study was to document AMR patterns in *Ec*O157 isolates from dairies in northern Colorado with a panel of antimicrobial agents commonly used on dairies and representative of medically important antimicrobial drug classes.

MATERIALS AND METHODS

E. coli O157 strains.

Three free-stall dairies located within a 20-mile (32.3-km) radius of Fort Collins, CO and representing a combined population of 2,750 lactating cattle were sampled monthly for 1 year (July 2013 through June 2014). During sampling, >10 g of feces was collected via rectal palpation from a convenience sample of cows within the first 21 days of lactation (939 fecal samples representing 899 cows) and cows culled on the day of sampling (104 fecal samples representing 104 cows). Samples were collected between 2 and 4 weeks apart, with 40 cows sampled twice during their first 21 days of lactation. Five cows were sampled both during early lactation and as culled cows. Life history features including parity, days in milk, and antimicrobial treatments were obtained for the current lactation from on-farm computer record systems (Dairy Comp 305, Valley Agricultural Software, Tulare, CA; DHI-Plus, DHI Computing Service, Provo, UT).

Each 10-g fecal sample was mixed 1:10 with buffered peptone water for both enrichment culture and direct plating. For direct plating, 100 µl was spread on selective sorbitol MacConkey agar with 5-bromo-4-chloro-3-indolyl-β-d-glucuronide (Oxoid Diagnostic Reagents, Basingstoke, England) containing 1.25 mg of potassium tellurite and 0.025 mg of cefixime (CT-SMAC-BCIG; HiMedia Laboratories, Mumbai, India). Plates were incubated at 37°C for 18 to 24 h (15). On plates containing >100 straw-colored colonies, suspect colonies were confirmed as positive for *Ec*O157 by agglutination using an *E. coli* O157 latex kit (Oxoid) following the manufacturer's instructions. Latex-positive isolated colonies were stored at −80°C in 10% sterile glycerol. Before the PCR assay, glycerol was removed from each isolate by centrifugation and then removal of the supernatant. The resulting pellet was resuspended in sterile molecular grade water.

The multiplex PCR assay was performed using previously published primers targeting the stx_1 and stx_2 genes (21) and the rfb_{O157} gene (31). All rfb_{O157} (and thus O157)-positive isolates, regardless of the presence of stx_1 and/or stx_2, were subsequently used in a PCR assay for the *eaeA* gene (21). The initial hot start PCR step of 5 min at 95°C is sufficient for lysing the bacteria; therefore, the cells were not prelysed before the PCR (10). Each 25-µl PCR consisted of 12.5 µl of master mix (multiplex PCR plus kit, Qiagen, Limburg, The Netherlands), 2.5 µl of primer mix containing 0.2 µM concentrations of each primer, 5 µl of molecular grade water, and 5 µl of bacterial culture as the direct template. The thermal cycling conditions consisted of an initial incubation step at 95°C for 5 min to activate the polymerase, 40 cycles of amplification with denaturation at 95°C for 30 s, annealing at 57°C for 90 s, and extension at 72°C for 30 s, and a final extension step at 68°C for 10 min. The PCR product was analyzed by agarose gel electrophoresis on a 2% agarose gel. A 100-bp molecular marker (Lonza Group Ltd., Basel, Switzerland) was added to the same gel to aid in the calculation of the size of the amplified DNA fragments.

The diluted fecal sample remaining after direct plating was incubated for 6 h at 37°C and stored overnight at 4°C. The enriched samples not confirmed as *Ec*O157 through direct plating (those not super shedding, i.e., with 0 to $<10^3$ to 10^4 CFU/g of feces) were subjected to immunomagnetic separation (IMS) using the Dyna-beads anti–*E. coli* O157 and

BeadRetriever System (Life Technologies, Oslo, Norway). IMS samples were subsequently plated onto CT-SMAC-BCIG and incubated for 18 to 24 h at 37°C. Suspect colonies were confirmed by latex agglutination and PCR targeting rfb_{O157}, stx_1, and stx_2. All rfb_{O157} (and thus O157)–positive isolates were subsequently subjected to the PCR assay for *eaeA* (21).

Antimicrobial susceptibility testing.

Bacterial isolates from frozen stocks were cultured on blood agar plates 24 h before susceptibility testing. The disk diffusion method was used with Mueller-Hinton agar (Difco, BD, Sparks, MD) and interpreted according to Clinical and Laboratory Standards Institute (Wayne, PA) 2006 recommendations using a Biomic V3 system (Giles Scientific, Santa Barbara, CA). *E. coli* (ATCC 25922 and ATCC 35218), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), and *Pseudomonas aeruginosa* (ATCC 27853) were used for quality control. *Ec*O157 isolates were tested for AMR to 10 antimicrobial agents active against gram-negative bacteria: amoxicillin–clavulanic acid (AMO), ampicillin (AMP), ceftiofur (CEF), enrofloxacin (ENR), florfenicol (FLO), streptomycin (STR), sulfisoxazole (SUL), tetracycline (TET), trimethoprim-sulfamethoxazole (TMS), and tulathromycin (TUL) (Sensi-Discs, Cockeysville, MD).

Statistical analysis.

The proportions of drug resistant isolates by dairy, virulence genes, parity, and antimicrobial treatments were compared using a chi-square test for equal proportions (PROC FREQ, SAS v. 9.4, SAS Institute, Cary, NC).

RESULTS

Seventy-five *Ec*O157 isolates were recovered from the three participating dairies. Of these isolates, 36 had one or both of the stx_1 and stx_2 genes (designated as EHEC) and 39 had the *eaeA* gene but neither the stx_1 or stx_2 gene (designated as aEPEC).

Seventy *Ec*O157 isolates, from 70 (7.5%) of the 939 samples, were derived from early lactation cows (i.e., <21 days in milk). Two nonresistant *Ec*O157 isolates were recovered from samples collected 2 weeks apart from the same early lactation cow. Five nonresistant *Ec*O157 isolates were obtained from 5 (4.8%) of the 104 cull cows. One nonresistant *Ec*O157 isolate, from 1 (0.1%) of the 1,043 total samples, was recovered from a super-shedding cow in early lactation.

Table 1 shows the AMR profiles of the isolates by dairy and virulence genes. Six isolates from six different cows were resistant to at least 1 of the 10 tested antimicrobials agents: four isolates were resistant to STR, SUL, and TET; one was resistant to STR and TET; and one was resistant to only TET. All of the resistant isolates were from a single dairy. The four isolates resistant to STR, SUL, and TET were EHECs. The other two isolates were aEPECs. One of the resistant EHEC isolates was recovered from a sample taken on 15 July 2013. The other resistant EHECs and the aEPECs were recovered from samples taken on 7 October 2013.

Comparisons of the proportion of resistant isolates by dairy revealed a significant difference between dairies ($P = 0.01$). For the dairy from which the resistant *Ec*O157 isolates were recovered, comparisons of the proportion of resistant strains by virulence genes ($P = 0.32$), parity ($P = 0.12$), and previous antimicrobial treatment during the lactation ($P = 0.15$) revealed no significant differences.

DISCUSSION

Overall, a low prevalence of AMR was observed (8%) in the 75 *Ec*O157 strains. In a previous study, AMR was found in 24% of *Ec*O157:H7 and *Ec*O157:NM strains obtained from animals, foods, and humans (17). In another study, 14% of *E. coli* O157:H7 strains from cattle carcasses, feces, hides, and ground beef were resistant to at least one antimicrobial agent (1).

Antimicrobial susceptibility testing in this study focused on drugs used on the participating dairies or agents representative of medically important antimicrobial drug classes. Penicillins (AMO and AMP), cephalosporins (CEF), fluoroquinolones (ENR), amphenicols (FLO), sulfonamides (SUL), tetracyclines (TET), trimethoprim-sulfamethoxazole (TMS), and macrolides (TUL) were used on all of the participating dairies. Aminoglycosides were not used on any of the dairies, but STR was tested as a representative aminoglycoside with a labeled use for cattle. Similar to our study, both Meng et al. (17) and Beier et al. (1) found that the most common AMR pattern among *Ec*O157 isolates was to STR, SUL, and TET. Similar AMR profiles involving STR, SUL, and TET have been documented in commensal *E. coli* and *Salmonella* across U.S. dairy operations in 21 states (14, 27).

Numerous differences were noted among the dairies in terms of biosecurity, expansion, facilities, environment, herd management, labor indices, and nutritional practices. All sampled animals in the study had been exposed to at least one class of antimicrobial agent at some point during their lifetime for production or therapeutic purposes. In our study, antimicrobial resistant *Ec*O157 isolates were detected on only one dairy. No significant effects on AMR profiles due to virulence genes, parity, or previous antimicrobial treatments within the current lactation period were detected.

AMR is conferred through specific biochemical mechanisms conveyed by resistance genes or factors (26). Multiple mechanisms of AMR can occur in a single isolate, leading to variable resistance patterns (16). Even low concentrations of drugs can select for resistant bacteria (23). The isolation of three EHECs with the same AMR profile on a single day on the same dairy highlights the potential for environmental contamination and subsequent pathogen spread at a given point in time. Many factors may have contributed to the *Ec*O157 AMR profiles at this dairy. The issue is how to prevent and control those pathogens that harbor AMR.

Appropriate health management practices can decrease the need for use of antimicrobial agents on the farm, thereby limiting animal exposure. Such practices may include the use of vaccines, probiotics, immune enhancers, good husbandry practices, and biosecurity. Education of animal producers and veterinarians regarding such strategies is essential for

preventing and controlling AMR (26). However, even with appropriate health management, cross-resistance can lead to resistance to a particular drug in bacteria that have not been exposed to that drug. For example, a heterogeneous increase in *E. coli* resistance to aminoglycosides (specifically STR) has been found in calves fed waste milk without aminoglycoside residues but with residues of other drugs (23). The apparent randomness of the bacterial AMR selection process argues for ongoing surveillance of AMR profiles in food-producing animals and foodborne pathogens.

As per the FDA veterinary feed directive final rule (30) announced in June 2015, an overarching goal of the FDA is to promote the judicious use of medically important antimicrobial agents in food-producing animals. Progress must be made toward eliminating production uses of medically important antimicrobial drugs and bringing the therapeutic uses of such drugs under the oversight of veterinarians (29). Limiting antimicrobial availability, implementing on-farm interventions, and enhancing surveillance are key strategies for reducing AMR in food animal agriculture (26). The results of this study provide background information for future comparative studies of AMR trends. Future studies should include more farms and more samples and should control for potential confounding factors of AMR that may underlie individual farm variation.

ACKNOWLEDGMENTS

This research was funded in part by the High Plains Intermountain Center for Agricultural Health and Safety (Fort Collins, CO). The authors are sincerely indebted to the owners and personnel of the three dairies for their invaluable collaboration during this study.

References

1. Beier RC, Poole TL, Brichta-Harhay DM, Anderson RC, Bischoff KM, Hernandez CA, Bono JL, Arthur TM, Nagaraja TG, Crippen TL, Sheffield CL, and Nisbet DJ. 2013. Disinfectant and antibiotic susceptibility profiles of Escherichia coli O157:H7 strains from cattle carcasses, feces, and hides and ground beef from the United States. J. Food Prot. 76:6–17. [PubMed: 23317851]
2. Centers for Disease Control and Prevention. 2015. National antimicrobial resistance monitoring system for enteric bacteria (NARMS). NARMS annual reports. Available at: http://www.cdc.gov/narms/reports/. Accessed 15 July 2015. [Google Scholar]
3. Cornick NA, Booher SL, and Moon HW. 2002. Intimin facilitates colonization by Escherichia coli O157:H7 in adult ruminants. Infect. Immun. 70:2704–2707. [PubMed: 11953416]
4. Dean-Nystrom EA, Bosworth BT, Cray WC, and Moon HW. 1997. Pathogenicity of Escherichia coli O157:H7 in the intestines of neonatal calves. Infect. Immun. 65:1842–1848. [PubMed: 9125570]
5. Ethelberg S, Olsen KEP, Scheutz F, Jensen C, Schiellerup P, Engberg J, Petersen AM, Olesen B, Gerner-Smidt P, and Mølbak K 2004. Virulence factors for hemolytic uremic syndrome, Denmark. Emerg. Infect. Dis. 10:842–847. [PubMed: 15200817]
6. European Centre for Disease Prevention and Control. 2015. Surveillance reports. Available at: http://ecdc.europa.eu/en/publications/surveillance_reports/Pages/index.aspx. Accessed 15 July 2015.
7. Farrokh C, Jordan K, Auvray F, Glass K, Oppegaard H, Raynaud S, Thevenot D, Condron R, De Reu K, Govaris A, Heggum K, Heyndrickx M, Hummerjohann J, Lindsay D, Miszczycha S, Moussiegt S, Verstraete K, and Cerf O. 2013. Review of Shiga-toxin–producing Escherichia coli (STEC) and their significance in dairy production. Int. J. Food Microbiol. 162:190–212. [PubMed: 22939912]
8. Ferens WA, and Hovde CJ. 2011. Escherichia coli O157:H7: animal reservoir and sources of human infection. Foodborne Pathog. Dis. 8:465–487. [PubMed: 21117940]

9. Fitzgerald AC, Edrington TS, Looper ML, Callaway TR, Genovese KJ, Bischoff KM, McReynolds JL, Thomas JD, Anderson RC, and Nisbet DJ. 2003. Antimicrobial susceptibility and factors affecting the shedding of E. coli O157:H7 and Salmonella in dairy cattle. Lett. Appl. Microbiol. 37:392–398. [PubMed: 14633110]
10. Fode-Vaughan KA, Maki JS, Benson JA, and Collins ML. 2003. Direct PCR detection of Escherichia coli O157:H7. Lett. Appl. Microbiol. 37:239–243. [Crossref] [Google Scholar] [PubMed: 12904226]
11. Friedrich AW, Bielaszewska M, Zhang W-L, Pulz M, Kuczius T, Ammon A, and Karch H. 2002. Escherichia coli harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J. Infect. Dis. 185:74–84. [PubMed: 11756984]
12. Gyles CL 2007. Shiga toxin–producing Escherichia coli: an overview. J. Anim. Sci. 85:E45–E62. [PubMed: 17085726]
13. Ikeda K, Ida O, Kimoto K, Takatorige T, Nakanishi N, and Tatara K. 1999. Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying Escherichia coli O157:H7 infection. Clin. Nephrol. 52:357–362. [PubMed: 10604643]
14. Lundin JI, Dargatz DA, Wagner BA, Lombard JE, Hill AE, Ladely SR, and Fedorka-Cray PJ. 2008. Antimicrobial drug resistance of fecal Escherichia coli and Salmonella spp. isolates from United States dairy cows. Foodborne Pathog. Dis. 5:7–19. [PubMed: 18260811]
15. March SB, and Ratnam S. 1986. Sorbitol-MacConkey medium for detection of Escherichia coli O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. 23:869–872. [PubMed: 3519658]
16. McDermott PF, Walker RD, and White DG. 2003. Antimicrobials: modes of action and mechanisms of resistance. Int. J. Toxicol. 22:135–143. [PubMed: 12745995]
17. Meng J, Zhao S, Doyle MP, and Joseph SW. 1998. Antibiotic resistance of Escherichia coli O157:H7 and O157:NM isolated from animals, food, and humans. J. Food Prot. 61:1511–1514. [PubMed: 9829195]
18. Moore DA, Smith DR, Sischo WM, Heaton K, and Besser TE. Escherichia coli O157:H7—discerning facts from fiction: an integrated research and extension project for multiple audiences. Zoonoses Public Health.
19. Mora A, Blanco JE, Blanco M, Alonso MP, Dhabi G, Echeita A, Gonzalez EA, Bernardez MI, and Blanco J. 2005. Antimicrobial resistance of Shiga toxin (verotoxin)–producing Escherichia coli O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbiol. 156:793–806. [PubMed: 15921895]
20. Nataro JP, and Kaper JB. 1998. Diarrheagenic Escherichia coli. Clin. Microbiol. Rev. 11:142–201. [PubMed: 9457432]
21. Paton AW, and Paton JC. 1998. Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. colihlyA, rfbO111, and rfbO157. J. Clin. Microbiol. 36:598–602. [PubMed: 9466788]
22. Pennington H 2010. Escherichia coli O157. Lancet 376:1428–1435. [PubMed: 20971366]
23. Pereira RV, Siler JD, Bicalho RC, and Warnick LD. 2014. In vivo selection of resistant E. coli after ingestion of milk with added drug residues. PLoS One 9:e115223.
24. Public Health Agency of Canada. 2015. CIPARS 2013—annual report. Canadian Integrated Program for Antimicrobial Resistance Surveillance. Available at: http://www.phac-aspc.gc.ca/cipars-picra/2013/annu-report-rapport-eng.php. Accessed 15 July 2015.
25. Rangel JM, Sparling PH, Crowe C, Griffin PM, and Swerdlow DL. 2005. Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982–2002. Emerg. Infect. Dis. 11:603–609. [PubMed: 15829201]
26. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 2007. Antimicrobial resistance issues in animal agriculture. C10.1299. Centers for Epidemiology and Animal Health, Fort Collins, CO.
27. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 2009. Dairy 2007: Salmonella and Campylobacter on U.S. dairy operations, 1996–2007. Centers for Epidemiology and Animal Health, Fort Collins, CO.

28. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 2012. Antimicrobial drug use and antimicrobial resistance on U.S. cow-calf operations, 2007–2008. Centers for Epidemiology and Animal Health, Fort Collins, CO.

29. U.S. Food and Drug Administration. 2013. Guidance for industry 213. New animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food-producing animals: recommendations for drug sponsors for voluntarily aligning product use conditions with GFI #209. U.S. Food and Drug Administration, Center for Veterinary Medicine, Silver Spring. MD.

30. U.S. Food and Drug Administration. 2015. Veterinary feed directive. Available at: https://www.federalregister.gov/articles/2015/06/03/2015-13393/veterinary-feed-directive. Accessed 3 July 2015.

31. Wang G, Clark CG, and Rodgers FG. 2002. Detection in Escherichia coli of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. J. Clin. Microbiol. 40:3613–3619. [PubMed: 12354854]

32. Wong CS, Jelacic S, Habeeb RL, Watkins SL, and Tarr PI. 2000. The risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. N. Engl. J. Med. 342:1930–1936. [PubMed: 10874060]

TABLE 1.

Antimicrobial resistance profiles for 75 EcO157 isolates from three Colorado dairies

Source, profile[a]	Total no. of isolates	No. of EcO157 isolates resistant to[b]:									
		AMO	AMP	CEF	ENR	FLO	STR	SUL	TET	TMS	TUL
Dairy											
A	33	0	0	0	0	0	5	4	6	0	0
B	33	0	0	0	0	0	0	0	0	0	0
C	9	0	0	0	0	0	0	0	0	0	0
Virulence profile											
EHEC	36	0	0	0	0	0	4	4	4	0	0
aEPEC	39	0	0	0	0	0	1	0	2	0	0

[a]EHEC. enterohemonhagic *Ec*0157 containing stx$_1$ and for stx$_2$ genes,aEPEC, enterohemonhagic *Ec*0157 containing the *eaeA* gene but no stx genes.

[b]AMO. amoxicillin-clavulanate; AMP, ampicillin; CEF, ceftiofur, ENR, enrofloxacin; FLO, florfenicol; STR, streptomyein; SUL, sulfonamides; TET, tetracycline; TMS, trimethoprim-sulfamethoxazole; TUL, tulathromyein. Four isolates were resistant to three antimierobial agents (STR, SUL, and TET), one isolate was resistant to two antimicrobial agents (STR and TET), and one isolate was resistant to one antimicrobial agent (TET).