



Research Paper

Diarrheagenic *Escherichia coli* with Multidrug Resistance in Cattle from Mexico

Yaraymi Ortiz¹, Brenda Cerino¹, Mauricio Moreno¹, Elizabeth Yañez¹, Norma Heredia¹, Jorge Dávila-Aviña¹, Teódulo Quezada², Alexandra Calle³, Santos García^{1,*}

¹ Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, San Nicolás de los Garza, Nuevo León, México 66455, Mexico

² Universidad Autónoma de Aguascalientes, Ciudad Universitaria, Aguascalientes, México 20100, Mexico

³ Texas Tech University, Amarillo, Texas 79106, USA

ARTICLE INFO

Keywords:

Antibiotic resistance

Cattle

Diarrheagenic

Escherichia coli

Pathotype

ABSTRACT

Mexico is an important producer/exporter of cattle and cattle products. In the last decade, an increase in antibiotic resistance in *E. coli* pathotype strains from livestock environments has been reported. This study aimed to determine the prevalence and antibiotic resistance profiles of *E. coli* pathotype strains from the feces of beef or dairy cattle reared in the states of Aguascalientes (AG, central) and Nuevo Leon (NL, northeastern) in Mexico. One hundred and ten fecal samples were collected (beef cattle-AG = 30; dairy cattle-AG = 20; beef cattle-NL = 30; dairy cattle-NL = 30). From these, *E. coli* was isolated using selective/differential media and confirmed on chromogenic media. Multiplex PCR was used to identify diarrheagenic *E. coli*, and the Kirby-Bauer technique was used to determine the antimicrobial susceptibilities. All the animals harbored *E. coli*, and pathotypes were found in 34 animals from both, beef and dairy cattle, mainly from Aguascalientes. Of the positive samples, 31 harbored a single *E. coli* pathotype, whereas three samples harbored two different pathotypes; EHEC was the most prevalent, followed by EPEC, ETEC, and EIEC or the combination of two of them in some samples. Most pathotype strains (19/37) were isolated from beef cattle. Neither the animals' productive purpose (beef or dairy cattle) ($r = 0.155$) nor the geographic regions (Aguascalientes or Nuevo Leon) ($r = -0.066$) had a strong positive correlation with the number of *E. coli* pathotype strains. However, animals reared in Aguascalientes had up to 8.5-fold higher risk of harboring *E. coli* pathotype strains than those reared in Nuevo Leon. All pathotype strains were resistant to erythromycin, tetracycline, and trimethoprim/sulfamethoxazole, and all dairy cattle pathotype strains were further resistant to five β -lactams (χ^2 , $P = 0.017$). The existence of these pathotypes and multidrug-resistant pathogens in the food chain is a risk to public health.

Introduction

Foodborne diseases in humans frequently occur due to the consumption of meat, milk, and derived products contaminated with livestock waste (Etcheverría & Padola, 2013). Although *E. coli* is part of the commensal microbiota in the intestines of warm-blooded animals, several strains cause health problems in humans and animals. These pathogenic organisms are grouped according to their virulence factors into six pathotypes: enterohemorrhagic (EHEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC),

enteroaggregative (EAEC), and diffusely adherent (DAEC) (Farfan et al., 2016).

EHEC, EPEC, and ETEC cause diarrhea in healthy and immunocompromised calves, affecting food production and trade (Soon et al., 2011). EIEC and atypical EAEC can be found in animals, including wild and domestic animals, especially swine (*Sus scrofa domestica*), sheep (*Ovis orientalis aries*), and cattle (*Bos taurus-indicus*). Studies in Europe show that the prevalence of EHEC ranges from 10% to 50% in healthy cattle (Oporto et al., 2008), and 29% to 82% in cattle farms in Germany (Geue et al., 2002); and 36% to 67% in sheep in Spain (Beutin

Abbreviations: BC, beef cattle; DC, Dairy cattle; AG, Aguascalientes; NL, Nuevo Leon.

* Corresponding author.

E-mail address: santos@microbiosymas.com (S. García).

<https://doi.org/10.1016/j.jfp.2024.100257>

Received 6 October 2023; Accepted 23 February 2024

Available online 28 February 2024

0362-028X/© 2024 The Author(s). Published by Elsevier Inc. on behalf of International Association for Food Protection.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

et al., 1993). Although few studies report on the presence of pathogenic *E. coli* in cattle for beef and milk production, Cobbaut et al. (2009) reported EHEC in 1.2% and 22.2% in dairy cattle and beef cattle, respectively, from Belgium.

The prevalence of diarrheagenic *E. coli* in animals is influenced by age, breed, gestation, feeding, productive purpose, climate, and cross-contamination (Renter & Sargeant, 2002). In the last 40 years, chicken, ground beef, and prepackaged raw meat-based foods have been commonly reported to be contaminated with diarrheagenic *E. coli* (Yang et al., 2017). Other related foods that are commonly contaminated include cheese and milk (with or without pasteurization), potatoes, leeks, lettuce, and preprepared soups involving a mixture of meat and vegetables (Yang et al., 2017; Luna et al., 2019).

The excessive use of antibiotics in livestock, as prophylactics or growth promoters, has contributed to the development of antibiotic resistance (Hosain et al., 2021). Prolonged exposure to low concentrations of antibiotics increases the selective pressure and facilitates the emergence of resistance in bacteria by mutations, horizontal gene transfer, and conjugation processes (Du et al., 2019). Foodborne diseases caused by *E. coli* worsen due to the critical dissemination of antibiotic-resistance genes, changing the antibiotic susceptibility patterns of *E. coli* and limiting the effectiveness of common antibiotics (Liebana et al., 2013).

Bok et al. (2015) reported high antibiotic resistance in *E. coli* pathotype strains in cattle feces in western Poland. Additionally, Shiga toxin-producing *E. coli* strains in dairy cattle have exhibited more resistance to antibiotics than those from beef cattle (Mashak, 2018).

Feces from livestock is a source of contamination not only for beef and beef products but also for produce (Hu et al., 2017). Mexico is the sixth most important producer/exporter of beef and beef products worldwide (2,130,521 tons per year) and the fifteenth most important bovine milk producer (13,239,778 tons per year) (Servicio de Información Agroalimentaria y Pesquera, 2022).

This study aimed to determine the prevalence and antibiotic resistance profiles of diarrheagenic *E. coli* isolated from the feces of beef and dairy cattle reared in two important animal breeding areas of Mexico, in central (Aguascalientes) and northeastern (Nuevo Leon) Mexico. Although several studies have been reported on *E. coli* pathotypes and antimicrobial resistance of isolates in Mexico, to the best of our knowledge, this is the first report that analyzes the antimicrobial resistance of bacteria according to the productive purpose of livestock. Also, this study analyzed the resistance to a greater number of antibiotics than those previously studied.

Materials and methods

Collection of fecal samples. Fecal samples were obtained from the rectal-anal region (Greenquist et al., 2005) from cattle with no sign of disease during spring of 2021. Samples were labeled according to the geographic region and productive purpose of the animals, as follows: cattle from the state of Aguascalientes used for beef production (BC-AG) or dairy production (DC-AG), and cattle from the state of Nuevo Leon used for beef production (BC-NL) or dairy production (DC-NL). A total of 110 fecal samples were collected (BC-AG $n = 30$; DC-AG $n = 20$; BC-NL $n = 30$; DC-NL $n = 30$). They were placed in sterile Nasco bags (500 g; Whirl Pak, Fort Atkinson, WI, USA) and kept at 2–8°C until processing.

In the case of cattle for meat production, samples were taken from livestock farms with $\approx 3,500$ (Aguascalientes) and $\approx 7,800$ animals (Nuevo Leon), divided into stables of 50–60 animals each. The animals were fed silage and a concentrate formulation plus zilpaterol hydrochloride (Virbac, Carros, France; 150 g per ton, Aguascalientes) or melengestrol acetate (Zoetis, Parsippany, New Jersey, USA; 0.25–0.50 mg per animal, Nuevo Leon) as growth promoters, per day. In the case of cattle for milk production, samples were taken from

farms with ≈ 80 (Aguascalientes) to ≈ 100 animals (Nuevo Leon). The animals were fed using a concentrate formulation with $\approx 18\%$ crude protein and a mixture of silages, alfalfa hay, and barley forage. The animals from Aguascalientes produced 23 or more liters of milk per cow per day, and those raised in Nuevo Leon ≈ 20 liters per cow per day.

Reference strains used as positive controls. The bacterial controls were EAEC serotype 042 (kindly provided by Dr. Fernando Navarro, CINVESTAV-Mexico), EHEC serotype O157:H7 ATCC 43894 (kindly provided by Dr. Lynne McLandsborough, Food Science Dept, University of Massachusetts, Amherst, MA, USA), and EPEC serotype 011 ATCC 43887, EIEC ATCC 43893, ETEC ATCC 35401, and *E. coli* ATCC 25922 (positive control for Clinical & Laboratory Standards Institute [CLSI] susceptibility tests) (CLSI, 2021) (kindly provided by Dr. Lee-Ann Jaykus, Dept of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC, USA).

The bacterial strains were preserved in tubes containing Brain Heart Infusion (BHI) broth (MCD LAB, Mexico) with 20% glycerol and stored at -80°C . Active cultures were prepared by transferring the strains into a tube containing 16 mL BHI agar (Neogen, Lansing, MI, USA) and incubating for 48 h at 37°C . Strains were stored at 4°C for no more than 8 weeks.

Isolation and microbiological identification of *E. coli*. For each fecal sample, 1 g feces was weighed, dissolved in a tube containing 9 mL phosphate-buffered saline (0.01 M; pH 7.2), vortex-homogenized for 1 min, streaked in four sectors of Petri dishes containing MacConkey (MCD LAB, Mexico) or MacConkey Sorbitol (Neogen, Lansing, MI, USA) agar, and incubated for 24 h at 37°C . Presumptive colonies of *E. coli* that fermented lactose on MacConkey agar (pink to red with bile salt precipitation halo; Fig. S1) were confirmed on the chromogenic agar Rapid *E. coli*2 (BioRad, Hercules, CA, USA, colonies purple to pink due to α -galactosidase and β -glucuronidase activities, Lauer et al., 2007). Presumptive colonies of *E. coli* O157 grown on MacConkey Sorbitol agar (small and pale yellow) were confirmed on CHROMagar O157 media (mauve color; Ile-de-France, Paris, France) (De Boer & Heuvelink, 2000).

All confirmed colonies were inoculated into tubes containing 5 mL BHI broth and incubated for 24 h at 37°C . Each culture was preserved with 20% glycerol (Sigma-Aldrich, San Luis, MO, USA) at -80°C (Revco Ux freezer; Thermo Scientific, Waltham, MA, USA).

Identification of diarrheagenic *E. coli* pathotypes. The five *E. coli* pathotypes were identified by their characteristic virulence genes: the presence of *eae* (which encoded the intimin adherence protein), *stx1* (Shiga toxin 1), and *stx2* (Shiga toxin 2) for EHEC; *eae* and *bfp* (bonding forming pilus) for EPEC, *lt* (thermo-labile toxin) and *stII* (thermo-stable toxin) for ETEC, *ipaH* (invasion plasmid antigen H) and *virF* (transcriptional regulator VirF) for EIEC, and *aafII* (aggregative adherence fimbriae type II) for EAEC. *E. coli* K-12 MG1655 was used as negative control. For template control, DNA was replaced by nuclease-free water.

E. coli isolates or controls were streaked on BHI agar plates and incubated for 24 h at 37°C . For each isolate or control, one colony was selected and placed into a 600- μL Eppendorf tube (Axygen, Union City, CA, USA) containing 15 μL lysis buffer comprising EDTA (10 mM; Sigma-Aldrich, San Luis, MO, USA), Tris HCL (100 mM; pH 7.4; Roche Diagnostics, Rotkreuz, Zug, Switzerland), NaCl (300 mM; Jalmek, Mexico), and Triton X-100 (2%; Sigma-Aldrich, San Luis, MO, USA). This mixture was vortex-homogenized for 1 min, boiled in a water bath for 10 min, and centrifuged (Accuspin Micro 17R, Thermo Scientific, Waltham, MA, USA) at 13,000 rpm for 10 min. The supernatant (DNA template) was transferred to a new tube and stored at -20°C (Godambe et al., 2017).

The integrity of the DNA template was determined using a UV-VIS spectrophotometer (NanoDrop 2000; Thermo Scientific, Waltham, MA, USA) at 260 nm. Multiplex Polymerase Chain Reaction (PCR)

was performed as described by Vidal et al. (2005) but using a different polymerase (MyTaq, Bioline, London, UK). The PCR mixture contained 10 µL of 5X reaction buffer, 5 µL (500 ng) DNA template, 0.4 µL oligonucleotides (Table S1) (Alpha DNA, Montreal, Quebec, Canada), 1 µL dNTP, and water (ddH₂O) to make the volume up to 50 µL. Positive, negative, and template controls (as mentioned above) were run on each multiplex PCR assay. The PCR amplification was performed in a Veriti Thermal Cycler (Applied Biosystems, Waltham, MA, USA) using the following parameters: 1 min at 94°C, followed by 40 cycles of 94°C for 1.5 min, primer annealing at 60°C for 1.5 min, extension at 72°C for 1.5 min, and a final extension at 72°C for 5 min.

Next, 10 µL PCR product was electrophoresed on 1.5% agarose (Green Research, Baton Rouge, LA, USA) gel, stained with Gel Red (Biotium, Fremont, CA, USA), visualized under UV light, and photographed (Kodac, Rochester, NY, USA).

Antimicrobial susceptibility of *E. coli* pathotype strains. The antimicrobial susceptibility of the *E. coli* pathotype strains to 17 antibiotics belonging to 8 major pharmacologic groups (Fig. S2) was determined using the Kirby–Bauer technique (Hudzicki, 2009). Each strain was grown in Mueller Hinton (MH) broth (Oxoid, Basingstoke, Hampshire, UK) at 37°C for 24 h and then adjusted to 0.5 McFarland standard ($\approx 1.5 \times 10^8$ CFU/ml). An aliquot was applied with a swab onto an MH agar plate (Bioxon, Becton-Dickinson, Mexico). Using sterile forceps, 17 antibiotic discs (BD-BBL; Becton Dickinson, Franklin Lakes, NJ, USA, except the colistin disk, which was from Oxoid, UK) were placed ≥ 10 mm from the edge of the plate with ≥ 30 mm between them. The plates were incubated at 37°C for 18 h, and then, the inhibition halos were measured. Resistant, intermediate, and susceptible strains were calculated using the breakpoints provided by the CLSI (2021).

In addition to the Kirby–Bauer technique, a colistin agar test was performed on presumptive colistin-resistant strains. The strains were activated and adjusted as described above. Next, 10 µL was inoculated and spread on the outer side of Petri dishes containing MH agar plus 1, 2, or 4 µg/mL colistin sulfate (Sigma-Aldrich, San Luis, MO, USA) and incubated at 37°C for 18 h. Strains with intermediate resistance grow on ≤ 2 µg/mL colistin and strains with resistance grow on ≥ 4 µg/mL colistin (CLSI, 2021).

Statistical analyses. The statistical analyses were performed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA). Origin 9.0 (OriginLab Corp., TX, USA) was used to create the graphics. χ^2 tests were used to determine whether the number of antibiotic resistances per *E. coli* pathotype strain differed significantly ($P \leq 0.05$) among DC, BC, AG, and NL samples. Furthermore, Pearson correlation coefficients were used to determine whether the number of *E. coli* pathotypes was correlated with productive purpose or geographical area, and whether positive animals and harboring a single *E. coli* pathotype were correlated. Lastly, odds ratios were calculated.

Results

Among the 110 samples, 440 colonies were isolated on MacConkey agar (4 colonies per sample), from which 299 were confirmed as *E. coli*

on Rapid *E. coli*2 agar, whereas 110 colonies (one per sample) were isolated from MacConkey Sorbitol agar as *E. coli* O157 presumptive, 28 colonies were confirmed on Chromagar O157 media (Fig. S1, Table S2). All 327 (of the 110 fecal samples) confirmed colonies were used to determine the prevalence of diarrheagenic *E. coli* by PCR (Table S1).

Multiplex PCR indicated that there were 37 diarrheagenic *E. coli* strains in 34/110 animals. The most prevalent pathotype was EHEC (21.8%, 24/110), followed by EPEC (6.3%, 7/110), ETEC (4.5%, 5/110), and EIEC (0.9%, 1/110). Among the positive samples, 91% (31/34) harbored a single pathotype strain (correlation between positive animals and harboring a single *E. coli* pathotype, $r = 1.00$), while 9% (3/34) harbored two pathotype strains (Table 1, Fig. 1).

BC samples mainly harbored one (17/19) pathotype strain; only two harbored two pathotype strains (EHEC + EPEC, EHEC + ETEC). Similarly, DC samples mainly harbored one pathotype strain (14/15); only one harbored two pathotype strains (EPEC + ETEC). Among the positive animals, there were more AG cattle (25/34) than NL cattle (9/34).

Regarding the productive purpose of the cattle (BC or DC), a slightly greater percentage of BC samples (35%; 21/60) than DC samples (32%; 16/50) had pathotype strains (Table 2). Regarding the BC samples, EHEC was detected in 23.3% (14/60), EPEC in 8.3% (5/60), and ETEC in 3.3% (2/60). Regarding the DC samples, EHEC was detected in 20% (10/50), EPEC in 4% (2/50), ETEC in 6% (3/50), and EIEC in 2% (1/50). EAEC was not detected in any sample. The correlation between the number of pathotype strains and the productive purpose (BC or DC) was weakly positive ($r = 0.155$); the odds ratio was 0.437 (95% CI: 0.105–1.817) and Phi was 0.249, indicating that the risk of harboring pathotype strains was independent of productive purpose.

EHEC, EPEC, and ETEC were detected in both regions (AG and NL), while EIEC was only detected in NL. The results showed that 75.7% (28/37) of the pathotype strains were from AG, and 24.3% (9/37) were from NL. There was no correlation between the number of pathotype strains and the geographic area (AG or NL) ($r = -0.066$), but the odds ratio was 2.288 (95% CI: 0.550–9.515) and Phi was 0.180, indicating that cattle from AG have an up to 8.5-fold increased risk of harboring pathotype strains than cattle from NL.

Various *E. coli* virulence genes were detected in the samples by multiplex PCR (Fig. 2). The *stx1* gene (encoding Shiga toxin I), which is usually detected in EHEC from humans and animals, was not detected in any sample, nor was the *aafII* gene (encoding aggregative adherence fimbriae type II). The BC samples had *eae* (9/21), *stx2* (5/21), *bfp* (5/21), *lt* (1/21), and the combination of *stII/lt* (1/21). The DC samples had *eae* (3/16), *stx2* (3/16), *bfp* (2/16), *stII* (3/16), and combinations of *stx2/eae* (4/16) and *virF/ipaH* (1/16). The latter finding was interesting because few reports highlight DC as reservoirs of EIEC. The results indicated that EHEC (mainly *stx2*-producing) was the most prevalent pathotype regardless of productive purpose.

Antimicrobial susceptibilities of *E. coli* pathotype strains. The 37 strains (21 from BC and 16 from DC) were assessed using the Kirby–Bauer technique. A larger percentage (91.9%) were resistant to

Table 1

Total number of *E. coli* pathotype-positive fecal samples of beef and dairy cattle in Aguascalientes or Nuevo Leon, Mexico

	BC-AG	DC-AG	Total AG	BC-NL	DC-NL	Total NL	Total BC	Total DC
Sampled animals	30	20	50	30	30	60	60	50
Animals harboring <i>E. coli</i> pathotypes	15	10	25	4	5	9	19	15
One strain/animal	13 ^a	9 ^b	22	4 ^c	5 ^d	9	17	14
Two strains/animal	2 ^e	1 ^f	3	-	-	-	2	1

The correlation between positive animals and harboring a single *E. coli* pathotype was a perfect linear positive association ($r = 1.000$).

Number of positive animals/*E. coli* pathotype(s) identified: ^a 9/EHEC, 3/EPEC, 1/ETEC; ^b 6/EHEC, 1/EPEC, 2/ETEC; ^c 3 EHEC, 1 EPEC; ^d 4 EHEC, 1 EIEC; ^e 1/ EHEC-EPEC, 1/EHEC-ETEC; ^f 1EPEC-ETEC.

BC = beef cattle; DC = dairy cattle; AG = Aguascalientes; NL = Nuevo Leon; EHEC = enterohemorrhagic *E. coli*; EPEC = enteropathogenic *E. coli*; ETEC = enterotoxigenic *E. coli*; EIEC = enteroinvasive *E. coli*.

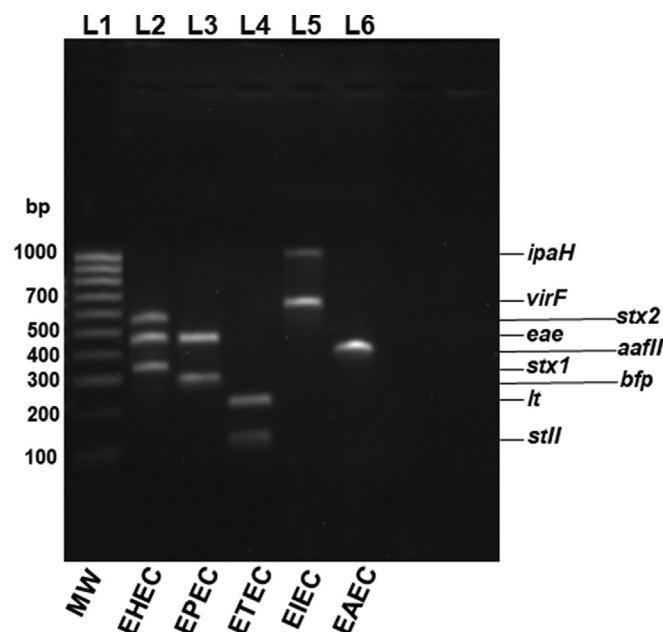


Figure 1. Agarose gel showing virulence genes of five *E. coli* pathotypes after multiplex PCR. Line 1, molecular weight marker; Line 2, EHEC (*eae* [482 bp], *stx1* [348 bp], and *stx2* [584 bp] genes); Line 3, EPEC (*eae* and *bfp* [300 bp] genes); Line 4, ETEC (*lt* [218 bp] and *stII* [129 bp] genes); Line 5, EIEC *ipaH* [933 bp] and *virF* [618 bp] genes); and Line 6, EAEC *aafII* (378 bp) gene.

β -lactams (such as ceftriaxone, cefazolin, penicillin, cephalothin, and cefuroxime) (86% [18/21] for BC vs. 100% [16/16] for DC) (Fig. 3, Table S3). Resistance to other β -lactams included cefepime (10% [2/21] for BC vs. 50% [8/16] for DC), cefoxitin (14% [3/21] for BC vs. 6% [1/16] for DC), and amoxicillin/clavulanic acid (10% [2/21] for BC vs. 19% [3/16] for DC).

Resistance to chloramphenicol (76% [16/21] for BC vs. 75% [12/16] for DC) was also notable. In addition, 100% of the strains were resistant to sulfamethoxazole/trimethoprim (sulfonamide), erythromycin (macrolide), and tetracycline.

Resistance to streptomycin (67% [14/21] for BC vs. 56% [9/16] for DC) and gentamicin (67% [14/21] for BC vs. 50% [8/16] for DC) was also observed. Resistance to tobramycin (33% [7/21]) and nitrofurantoin (5% [1/21]) was observed among the BC samples but not the DC samples. Presumptive resistance to colistin (using SensiDiscs) was detected in 11% (4/37) of the strains (14% [3/21] for BC vs. 6% [1/16] for DC) but no resistance was confirmed based on the colistin agar test (CLSI, 2021) (Fig. S3).

The χ^2 test showed that the number of antibiotic resistances per pathotype strain differed significantly among DC samples ($P = 0.017$) but not BC samples ($P = 0.533$) and among AG samples ($P = 0.004$) but not NL samples ($P = 0.501$).

Our results showed that there was a broad antibiotic resistance profile in strains from DC, as 100% were resistant to ceftriaxone, cefazolin, penicillin, cephalothin, cefuroxime, amoxicillin/clavulanic acid, erythromycin, tetracycline, and sulfamethoxazole/trimethoprim. This may partly be the result of β -lactamase causing cross-resistance to β -lactams (central graphic in Fig. 2).

Discussion

For many years, cattle have been a primary source of meat and milk for humanity. However, these animals can be reservoirs of antibiotic-resistant foodborne pathogens for humans. Therefore, the detection, identification, and determination of antibiotic resistance of pathogens in cattle are essential to ensure the safety of the foods provided by the livestock industry (Rajeev et al., 2017).

In 2021, Mexico was the sixth and fifteenth largest global exporter of beef and beef milk, respectively, which led us to monitor the prevalence of diarrheagenic *E. coli* and its antibiotic resistance in cattle raised in two important reproductive regions of Mexico. A careful bibliographical analysis of the studies published on *E. coli* pathotypes in Mexico, from 1987 to 2021, found a total of 63 publications. Of these, only 14 analyzed fecal samples from cattle in Mexico (including fecal samples from slaughterhouses or farms), where only six analyzed antimicrobial susceptibility against a very limited number of antibiotics. In addition, these reports focused on EHEC and analyzed cattle carcasses and environmental samples such as water, soil, and animal feeds.

This study determined the prevalence and the resistant profiles of *E. coli* pathotypes in fecal samples of BC and DC reared in central (Aguascalientes) and northeastern (Nuevo Leon) Mexico, two important cattle breeding areas of Mexico. Samples were taken directly from the rectal-anal region, as described by Greenquist et al. (2005); this is recommended for the detection of *E. coli* pathotypes instead of direct microbiological analysis of feces, as bacteria colonizing an animal's intestine are not necessarily released when the animal defecates.

Although serotyping using somatic (O), flagellar (H), and capsular (K) antigens is one system to classify *E. coli* strains, the detection of specific targets (i.e., virulence genes) is the commonest way to differentiate among pathotypes (Vidal et al., 2005; Stenutz et al., 2006). For this, multiplex PCR was used to determine the prevalence of *E. coli* pathotypes, indicating that 35% of BC samples vs. 32% of DC samples had diarrheagenic strains. EHEC exhibited the highest prevalence (23.3% for BC vs. 20% for DC), followed by EPEC (8.3% for BC vs. 4% for DC), ETEC (3.3% for BC vs. 6% for DC), and EIEC (2% for DC). AG samples had a higher prevalence of positive samples than NL samples.

Sobhy et al. (2020) also found that EHEC was the most frequent pathotype in bovine rectal samples in Egypt. EHEC was more common in BC than DC in the USA states of Michigan (21% for BC vs. 13% for DC) (Venegas et al., 2016), Washington (0.33% for BC vs. 0.28% for DC) (Hancock et al., 1994), and New York (7.1% for BC vs. 1.3%

Table 2

Prevalence of *E. coli* pathotypes isolated from fecal samples of beef and dairy cattle in Aguascalientes or Nuevo Leon, Mexico

<i>E. coli</i> pathotype	Number of <i>E. coli</i> pathotype strains (%)							
	BC-AG	DC-AG	Total AG	BC-NL	DC-NL	Total NL	Total BC	Total DC
EHEC	11 (22)	6 (12)	17 (34)	3 (5)	4 (6.7)	7 (11.7)	14 (23.3)	10 (20)
EPEC	4 (8)	2 (4)	6 (12)	1 (1.7)	–	1 (1.7)	5 (8.3)	2 (4)
ETEC	2 (34)	3 (6)	5 (10)	–	–	–	2 (3.3)	3 (6)
EIEC	–	–	–	–	1 (1.7)	1 (1.7)	–	1 (2)
Total	17 (34)	11 (22)	28 (56)	4 (6.7)	5 (8.3)	9 (15)	21 (35)	16 (32)

There was no correlation ($r = -0.066$) between the number of *E. coli* pathotype strains and geographic area, but a weakly positive correlation ($r = 0.155$) between the number of *E. coli* pathotype strains and productive purpose.

BC = beef cattle; DC = dairy cattle; AG = Aguascalientes; NL = Nuevo Leon; (–) = not detected; EHEC = enterohemorrhagic *E. coli*; EPEC = enteropathogenic *E. coli*; ETEC = enterotoxigenic *E. coli*; EIEC = enteroinvasive *E. coli*.

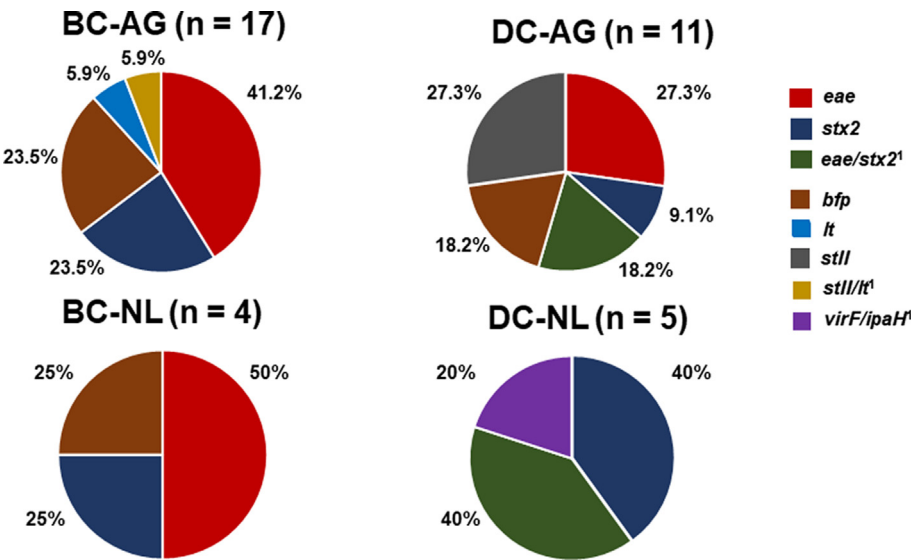


Figure 2. Abundance of virulence genes in *E. coli* pathotype strains isolated from fecal samples of beef and dairy cattle in Aguascalientes or Nuevo Leon, Mexico. ¹Genes detected simultaneously in the *E. coli* pathotype strains. Virulence genes used to identify *E. coli* pathotypes: *eae/stx1/stx2* (EHEC); *eae/bfp* (EPEC); *stII/lt* (ETEC); *virF/ipaH* (EIEC); *aafII* (EAEC); *daaE* (DAEC). BC = beef cattle; DC = dairy cattle; AG = Aguascalientes; NL = Nuevo Leon.

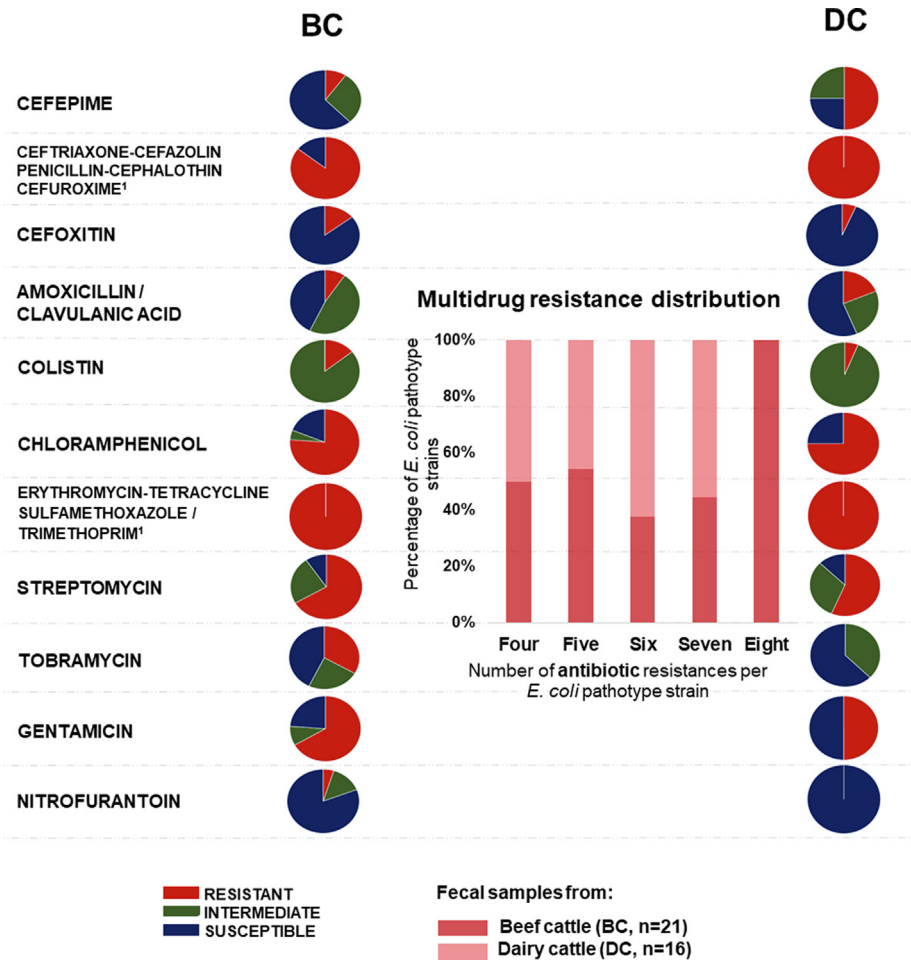


Figure 3. Distribution of antimicrobial susceptibilities among *E. coli* pathotype strains isolated from fecal samples of beef and dairy cattle in Aguascalientes or Nuevo Leon, Mexico. The strains were classified as Resistant (red) (CLSI, 2021), Intermediate (green), or Susceptible (blue). The central graphic shows the number of antibiotics (4–8) that strains with multidrug resistance could resist. Number of antibiotic resistances per *E. coli* pathotype strain differed significantly among DC samples ($\chi^2 P = 0.017$) but not among BC samples ($P = 0.533$). Resistance to β lactams were considered as one. ¹*E. coli* pathotype strains with identical results (i.e., all resistant) for several antibiotics based on susceptibility testing. BC = beef cattle; DC = dairy cattle.

for DC) (McDonough et al., 2000; Reinstein et al., 2007). However, EHEC was only found in DC in Brazil (up to 18.9%) (Vicente et al., 2005) and Argentina (3.8%) (Tanaro et al., 2010) and not in BC.

Callaway et al. (2004) found a high frequency of EHEC in fecal samples from DC and BC during the spring-summer seasons in central Mexico, which coincides with the higher frequency of bacterial shedding among cattle there. Navarro et al. (2018) also found that EHEC was the most frequent pathotype in fecal samples from DC in the pacific region of Mexico, followed by ETEC, EPEC, and EIEC, which is similar to the pathotype diversity in our DC samples from NL. Our results confirmed that cattle (BC and DC) can act as reservoirs of *E. coli* pathotypes (irrespective of factors such as productive purpose and geographical area). BC from AG had the highest prevalence of *E. coli* pathotypes, while DC from NL had greater diversity. Although EHEC was more prevalent, EPEC, ETEC, and EIEC were also detected.

Although *E. coli* pathotypes appear to be widespread, these can be sporadic and highly dependent on season (Callaway et al., 2004). A monthly test of beef herds in Germany revealed STEC prevalence rates of up to 80% in summer (Geue et al., 2002). Cobbold et al. (2004) in the United States reported a higher prevalence of STEC (9%) during fall compared to winter. Cobbaut et al., (2009) reported that summer was the season with the highest prevalence of *E. coli* O157 in Belgium, whereas Barkocy et al. (2003) found *E. coli* O157:H7 and non-O157 STEC in more fecal samples in the spring compared to other seasons in the United States, coinciding with our sampling period. In addition, habitat, and environmental factors such as limited space and anthropogenic pressure can lead to changes in bacterial prevalence, while open pastures also provides the opportunity to spread these pathogens (Bok et al., 2015). Although our study was carried out in important animal breeding areas of Mexico during the spring season, considering the variations in the geographic and seasonal fluctuation of *E. coli* pathotypes observed in other regions, additional studies involving different seasons, animal management, and environmental conditions are required to know the prevalence of *E. coli* pathotypes in those conditions.

Antimicrobial resistance of pathogens reduces treatment options for humans and animals. *E. coli* often resists the most widely used antibiotics, partly due to its capacity to incorporate exogenous DNA (such as resistance genes), mainly by horizontal transfer (Wellington et al., 2013). Using the Kirby–Bauer disk diffusion technique, we found that 95.1% of the *E. coli* pathotype strains resisted at least four β -lactams. This suggests cross-resistance due to β -lactamases, which are able to hydrolyze β -lactam rings (Gelalcha & Kerro, 2022).

The four presumptive colistin-resistant strains found in this study were subsequently confirmed to be nonresistant by the colistin agar test; however, as colistin resistance has been found in *E. coli* strains from the Mexican agroenvironment (Pérez-Garza et al., 2021), we recommend testing for this antibiotic resistance in future studies. In China, *E. coli* with this resistance were confirmed in bovine feces (Zhang et al., 2019), and strains with *mcr*-dependent or independent mechanisms were detected. Yamamoto et al. (2019) suggested that the prevalence of colistin resistance may be due to the prophylactic use of polymyxins (the colistin pharmacological group) in animal feed. Notably, the three presumptive colistin-resistant strains from BC were sensitive to all eight β -lactams tested, and they were the only strains that remained sensitive to this antibiotic group.

In this study, all the *E. coli* pathotype strains were resistant to erythromycin, tetracycline, and trimethoprim/sulfamethoxazole. A previous study reported that ~90% of *E. coli* strains from DC fecal samples were resistant to erythromycin and tetracycline (Sobur et al., 2019). Notably, the use and abuse of tetracycline as a prophylactic in livestock environments can lead to the proliferation, accumulation, and widespread distribution of antibiotic-resistant bacteria and their related genes (Du et al., 2019). Trimethoprim/sulfamethoxazole resistance was also reported in all *E. coli* strains from DC with mastitis in Jordan (Ismail & Abutarbush, 2020). Similarly, in Iran, high resistance

(90.3%) of *E. coli* strains from the feces of calves was reported (Shahrani et al., 2014). The high resistance to sulfonamides (such as sulfamethoxazole) is probably a consequence of 50 years of continuous use. As expected, other drugs with limited use in cattle, such as tobramycin, exhibited low resistance (19%, 7/37).

In conclusion, this study demonstrated that cattle from the studied regions are reservoirs of multidrug-resistant diarrheagenic *E. coli*. Among the 110 fecal samples from BC and DC reared in AG and NL in Mexico, 31% (34/110) were positive for one or more diarrheagenic *E. coli* pathotypes. The most common pathotype identified was EHEC (21.8%; 24/110; *stx2/eae*), followed by EPEC (6.3%; 7/110, *bfp*), ETEC (4.3%; 5/110, *lt/stII*), and EIEC (0.9%; 1/110 *ipaH/virF*). There were more BC (19/34) than DC (15/34) samples that were positive for *E. coli* pathotypes, and there were more AG (25/34) than NL (9/34) samples that were positive. Among the *E. coli* pathotype strains, we observed dangerous levels of resistance (100%) to erythromycin, trimethoprim/sulfamethoxazole, and tetracycline, which are the frequently used antibiotics in livestock environments. Notably, 100% of strains from DC samples were resistant to five β -lactams, probably due to the frequent β -lactam use during deliveries and for mastitis.

Multidrug-resistant diarrheagenic *E. coli* is a biological hazard for farmworkers and when found as a contaminant in foods. Efforts to control these pathogens are necessary to avoid their presence in farm and food production environments and on the consumer table.

CRedit authorship contribution statement

Yaraymi Ortiz: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis. **Brenda Cerino:** Methodology, Investigation. **Mauricio Moreno:** Methodology, Investigation. **Elizabeth Yañez:** Methodology, Investigation, Formal analysis. **Norma Heredia:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Jorge Dávila-Aviña:** Writing – review & editing, Resources, Methodology, Investigation. **Teóduelo Quezada:** Writing – review & editing, Writing – original draft, Validation, Resources, Formal analysis. **Alexandra Calle:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition. **Santos García:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the Consejo Nacional de Ciencia y Tecnología de México (A1-S-25033).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jfp.2024.100257>.

References

- Barkocy, G. A., Arthur, T. M., Rivera-Betancourt, M., Nou, X., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2003). Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *Journal of Food Protection*, 66, 1978–1986. <https://doi.org/10.4315/0362-028x-66.11.1978>.
- Beutin, L., Geier, D., Steinrück, H., Zimmermann, S., & Scheut, F. (1993). Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in

- seven different species of healthy domestic animals. *Journal of Clinical Microbiology*, 31, 2483–2488. <https://doi.org/10.1128/jcm.31.9.2483-2488.1993>.
- Bok, E., Mazurek, J., Stosik, M., Wojciech, M., & Baldy-Chudzik, K. (2015). Prevalence of virulence determinants and antimicrobial resistance among commensal *Escherichia coli* derived from dairy and beef cattle. *International Journal of Environmental Research and Public Health*, 12, 970–985. <https://doi.org/10.3390/ijerph120100970>.
- Callaway, T. R., Anderson, R. C., Tellez, G., Rosario, C., Nava, G. M., Eslava, C., Blanco, M. A., Quiroz, M. A., Olguín, A., Herradora, M., Edrington, T. S., Genovese, J. K., Harvey, R. B., & Nisbet, D. J. (2004). Prevalence of *Escherichia coli* O157 in cattle and swine in Central Mexico. *Journal of Food Protection*, 67, 2274–2276. <https://doi.org/10.4315/0362-028X-67.10.2274>.
- CLSI (Clinical and Laboratory Standards Institute). (2021). Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100-Ed31. Wayne, PA, USA.
- Cobbaut, K., Berkvens, D., Houf, K., De Deken, R., & De Zutter, L. (2009). *Escherichia coli* O157 prevalence in different cattle farm types and identification of potential risk factors. *Journal of Food Protection*, 72, 1848–1853. <https://doi.org/10.4315/0362-028X-72.9.1848>.
- Cobbold, R. N., Rice, D. H., Szymanski, M., Call, D. R., & Hancock, D. D. (2004). Comparison of shiga-toxinogenic *Escherichia coli* prevalences among dairy, feedlot, and cow-calf herds in Washington State. *Applied and Environmental Microbiology*, 70, 4375–4378. <https://doi.org/10.1128/AEM.70.7.4375-4378.2004>.
- De Boer, E., & Heuvelink, A. E. (2000). Methods for the detection and isolation of Shiga toxin-producing *Escherichia coli*. Symposium supplement. *Journal of Applied Microbiology*, 88, 133S–143S. <https://doi.org/10.1111/j.1365-2672.2000.tb05341.x>.
- Du, B., Yang, Q., Wang, R., Wang, R., Wang, Q., & Xin, Y. (2019). Evolution of antibiotic resistance and the relationship between the antibiotic resistance genes and microbial compositions under long-term exposure to tetracycline and Sulfamethoxazole. *International Journal of Environmental Research and Public Health*, 16, 4681–4696. <https://doi.org/10.3390/ijerph16234681>.
- Etcheverría, A. I., & Padola, N. L. (2013). Shiga toxin-producing *Escherichia coli*. *Virulence*, 4, 366–372. <https://doi.org/10.4161/viru.24642>.
- Farfan, A. E., Ariza, S. C., Vargas, F. A., & Vargas, L. V. (2016). Virulence mechanisms of enteropathogenic *Escherichia coli*. *Chilean Journal of Infectology*, 33, 438–450. <https://doi.org/10.4067/S0716-10182016000400009>.
- Gelachla, B. D., & Kerro, O. (2022). Extended-spectrum beta-lactamases producing Enterobacteriaceae in the USA dairy cattle farms and implications for public health. *Antibiotics*, 11, 1313. <https://doi.org/10.3390/antibiotics11101313>.
- Geue, L., Segura, M., Conraths, F. J., Kuczius, T., Bockemühl, J., Karch, H., & Gallien, P. (2002). A long-term study on the prevalence of shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. *Epidemiology & Infection*, 129, 173–185. <https://doi.org/10.1017/S0950268802007288>.
- Godambe, L. P., Bandekar, J., & Shashidhar, R. (2017). Species specific PCR based detection of *Escherichia coli* from Indian foods. 3 *Biotech*, 7. <https://doi.org/10.1007/s13205-017-0784-8>.
- Greenquist, M. A., Drouillard, J. S., Sargeant, J. M., Depenbusch, B. E., Shi, X., Lechtenberg, K. F., & Nagaraja, T. G. (2005). Comparison of rectoanal mucosal swab cultures and fecal cultures for determining prevalence of *Escherichia coli* O157:H7 in feedlot cattle. *Applied and Environmental Microbiology*, 71, 6431–6433. <https://doi.org/10.1128/AEM.71.10.6431-6433.2005>.
- Hancock, D. D., Besser, T. E., Kinsel, M. L., Tarr, P. I., Rice, D. H., & Paros, M. G. (1994). The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. *Epidemiology & Infection*, 113, 199–207. <https://doi.org/10.1017/S0950268800051633>.
- Hosain, M. Z., Kabir, S. M. L., & Kamal, M. M. (2021). Antimicrobial uses for livestock production in developing countries. *Veterinary World*, 14, 210–221. <https://doi.org/10.14202/vetworld.2021.210-221>.
- Hu, Y., Cheng, H., & Tao, S. (2017). Environmental and human health challenges of industrial livestock and poultry farming in China and their mitigation. *Environment International*, 107, 111–130. <https://doi.org/10.1016/j.envint.2017.07.003>.
- Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. Retrieved January 25, 2024, from <https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-DiffusionSusceptibility-Test-Protocol-pdf>
- Ismail, Z. B., & Abutarbush, S. M. (2020). Molecular characterization of antimicrobial resistance and virulence genes of *Escherichia coli* isolates from bovine mastitis. *Veterinary World*, 13, 1588–1593. <https://doi.org/10.14202/vetworld.2020.1588-1593>.
- Lauer, W. F., Martinez, F. L., & Patel, A. (2007). Validation of RAPID'E. coli 2 for enumeration and differentiation of *Escherichia coli* and other coliform bacteria in selected foods. *Journal of AOAC International*, 90, 1284–1315. <https://doi.org/10.1093/jaoac/90.5.1284>.
- Liebauna, E., Carattoli, A., Coque, T. M., Hasman, H., Magiorakos, A. P., Mevius, D., Peixe, L., Poirel, L., Schuepbach, G., Torneke, K., Torren, J., Torres, C., & Threlfall, J. (2013). Public health risks of enterobacterial isolates producing extended-spectrum β -lactamases or AmpC β -lactamases in food and food-producing animals: An EU perspective of epidemiology, analytical methods, risk factors, and control options. *Clinical Infectious Diseases*, 56, 1030–1037. <https://doi.org/10.1093/cid/cis1043>.
- Luna, J. J., Arenas, M. M. P., Martínez, C., Silva, J., & Luna, M. L. (2019). The Role of pathogenic *E. coli* in fresh vegetables: behavior, contamination factors, and preventive measures. *International Journal of Microbiology*, 2019. <https://doi.org/10.1155/2019/2894328>.
- Mashak, Z. (2018). Virulence genes and phenotypic evaluation of the antibiotic resistance of vero toxin producing *Escherichia coli* recovered from milk, meat, and vegetables. *Jundishapur Journal of Microbiology*, 11, e62288. <https://doi.org/10.5812/jjm.62288>.
- McDonough, P. L., Rossiter, C. A., Rebhun, R. B., Stehman, S. M., Lein, D. H., & Shin, S. J. (2000). Prevalence of *Escherichia coli* O157:H7 from cull dairy cows in New York state and comparison of culture methods used during preharvest food safety investigations. *Journal of Clinical Microbiology*, 38, 318–322. <https://doi.org/10.1128/jcm.38.3.318-322.2000>.
- Navarro, A., Cauich, P. I., Trejo, A., Gutiérrez, A., Díaz, S. P., Díaz, M., Cravioto, A., & Eslava, C. (2018). Characterization of diarrheagenic strains of *Escherichia coli* isolated from cattle raised in three regions of Mexico. *Frontiers in Microbiology*, 9. <https://doi.org/10.3389/fmicb.2018.02373>.
- Oporto, B., Esteban, J. I., Aduriz, G., Juste, R. A., & Hurtado, A. (2008). *Escherichia coli* O157:H7 and non-O157 shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in northern Spain. *Zoonoses and Public Health*, 55, 73–81. <https://doi.org/10.1111/j.1863-2378.2007.01080.x>.
- Pérez, J., Franco, E., García, A., García, S., Leon, J. S., Jaykus, L. A., & Heredia, N. (2021). The cantaloupe farm environment has a diverse genetic pool of antibiotic-resistance and virulence genes. *Foodborne Pathogens and Disease*, 18, 469–476. <https://doi.org/10.1089/fpd.2020.2900>.
- Rajeev, M., Mutinda, M., & Ezenwa, V. O. (2017). Pathogen exposure in cattle at the livestock-wildlife interface. *EcoHealth*, 14, 542–551. <https://doi.org/10.1007/s10393-017-1242-0>.
- Reinstein, S., Fox, J. T., Shi, X., & Nagaraja, T. G. (2007). Prevalence of *Escherichia coli* O157:H7 in gallbladders of beef cattle. *Applied and Environmental Microbiology*, 73, 1002–1004. <https://doi.org/10.1128/AEM.02037-06>.
- Renter, D. G., & Sargeant, J. M. (2002). Enterohemorrhagic *Escherichia coli* O157: Epidemiology and ecology in bovine production environments. *Animal Health Research Reviews*, 3, 83–94. <https://doi.org/10.1079/ahr.200245>.
- Servicio de Información Agroalimentaria y Pesquera. (2022). Panorama Agroalimentario de México. Recuperado Agosto 29, 2023, de <https://online.pubhtml5.com/aei/ygryd/#p=158>.
- Shahrani, M., Dehkordi, F. S., & Momtaz, H. (2014). Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. *Biological Research*, 47. <https://doi.org/10.1186/0717-6287-47-28>.
- Sobhy, N. M., Yousef, S. G. A., Aboubakr, H. A., Nisar, M., Nagaraja, K. V., Mor, S. K., Valeris-Chacin, R. J., & Goyal, S. M. (2020). Virulence factors and antibiograms of *Escherichia coli* isolated from diarrheic calves of Egyptian cattle and water buffaloes. *PLOS ONE*, 15, e0232890. <https://doi.org/10.1371/journal.pone.0232890>.
- Sobur, A., Sabuj, A. A. M., Sarker, R., Taufiqur, A. M. M., Lutful, S. M., & Rahman, T. (2019). Antibiotic-resistant *Escherichia coli* and *Salmonella* spp. associated with dairy cattle and farm environments having public health significance. *Veterinary World*, 12, 984–993. <https://doi.org/10.14202/vetworld.2019.984-993>.
- Soon, J. M., Chadd, S. A., & Baines, R. N. (2011). *Escherichia coli* O157:H7 in beef cattle: On farm contamination and pre-slaughter control methods. *Animal Health Research Reviews*, 12, 197–211. <https://doi.org/10.1017/S1466252311000132>.
- Stenutz, R., Weintraub, A., & Widmalm, G. (2006). The structures of *Escherichia coli* O-polysaccharide antigens. *FEMS Microbiology Reviews*, 30, 382–403. <https://doi.org/10.1111/j.1574-6976.2006.00016.x>.
- Tanaro, J. D., Leotta, G. A., Lound, L. H., Galli, L., Piaggio, M. C., Carbonari, C. C., Araujo, S., & Rivas, M. (2010). *Escherichia coli* O157 in bovine feces and surface water streams in a beef cattle farm of Argentina. *Foodborne Pathology and Disease*, 7, 475–479. <https://doi.org/10.1089/fpd.2009.0431>.
- Venegas, C., Henderson, S., Khare, A., Mosci, R. E., Lehnert, J. D., Singh, P., Ouellette, L. M., Norby, B., Funk, J. A., Rust, S., Bartlett, P. C., Grooms, D., & Manning, S. D. (2016). Factors associated with shiga toxin-producing *Escherichia coli* shedding by dairy and beef cattle. *Applied and Environmental Microbiology*, 82, 5049–5056. <https://doi.org/10.1128/AEM.00829-16>.
- Vicente, H. I. G., Amaral, L. A., & Cerqueira, A. M. F. (2005). Shiga-toxinogenic *Escherichia coli* serogroups O157, O111 and O113 in feces, water and milk samples from dairy farms. *Brazilian Journal of Microbiology*, 36, 217–222. <https://doi.org/10.1590/S1517-83822005000300003>.
- Vidal, M., Kruger, E., Durán, C., Lagos, R., Levine, M., Prado, V., Toro, C., & Vidal, R. (2005). Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. *Journal of Clinical Microbiology*, 43, 5362–5365. <https://doi.org/10.1128/JCM.43.10.5362-5365.2005>.
- Wellington, E. M. H., Boxall, A. B. A., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., Johnson, A. S., Jones, D. L., Lee, N. M., Otten, W., Thomas, C. M., & Williams, A. P. (2013). The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *The Lancet Infectious Diseases*, 13, 155–165. [https://doi.org/10.1016/S1473-3099\(12\)70317-1](https://doi.org/10.1016/S1473-3099(12)70317-1).
- Yamamoto, Y., Calvopina, M., Izurieta, R., Villacres, I., Kawahara, R., Sasaki, M., & Yamamoto, M. (2019). Colistin-resistant *Escherichia coli* with mcr genes in the livestock of rural small-scale farms in Ecuador. *BMC Research Notes*, 12, 121. <https://doi.org/10.1186/s13104-019-4144-0>.
- Yang, S. C., Lin, C. H., Aljuffali, I. A., & Fang, J. Y. (2017). Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. *Archives of Microbiology*, 199, 811–825. <https://doi.org/10.1007/s00203-017-1393-y>.
- Zhang, X., Zhang, B., Guo, Y., Wang, J., Zhao, P., Liu, J., & He, K. (2019). Colistin resistance prevalence in *Escherichia coli* from domestic animals in intensive breeding farms of Jiangsu Province. *International Journal of Food Microbiology*, 291, 87–90. <https://doi.org/10.1016/j.ijfoodmicro.2018.11.013>.