

# Antibiotic-resistant *E. coli* in surface water and groundwater in dairy operations in Northern California

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**Abstract** Generic *Escherichia coli* was isolated from surface water and groundwater samples from two dairies in Northern California and tested for susceptibility to antibiotics. Surface samples were collected from flush water, lagoon water, and manure solids, and groundwater samples were collected from monitoring wells. Although *E. coli* was ubiquitous in surface samples with concentrations ranging from several hundred thousand to over a million colony-forming units per 100 mL of surface water or per gram of surface solids, groundwater under the influence of these high surface microbial loadings had substantially fewer bacteria (3- to 7-log<sub>10</sub> reduction). Among 80 isolates of *E. coli* tested, 34 (42.5 %) were resistant to one or more antibiotics and 22 (27.5 %) were multi-antibiotic resistant (resistant to  $\geq 3$  antibiotics), with resistance to tetracycline, cefoxitin, amoxicillin/clavulanic acid, and ampicillin being the most common. *E. coli* isolates from the calf hutch area exhibited the highest levels of multi-antibiotic resistance, much higher than isolates in surface soil solids from heifer and cow pens, flush alleys,

manure storage lagoons, and irrigated fields. Among *E. coli* isolates from four groundwater samples, only one sample exhibited resistance to ceftriaxone, chloramphenicol, and tetracycline, indicating the potential of groundwater contamination with antibiotic-resistant bacteria from dairy operations.

**Keywords** Dairy · Water · *E. coli* · Antibiotic resistance

## Introduction

Dairy cattle are natural hosts of several food-borne and waterborne bacterial pathogens, and persistent fecal shedding of bacteria has been well documented in dairy operations: A survey of the prevalence of *Salmonella* in dairy cattle in New York state revealed 77 % (44 out of 57) dairy herds produced *Salmonella*-positive fecal samples (Cummings et al. 2010). In a study conducted in Ohio, *Campylobacter jejuni*, *Salmonella*, and *Escherichia coli* O157:H7 were isolated in 7 % (48 out of 686), 6.7 % (39 out of 585), and 2.1 % (21 out of 1,026) of fecal samples, respectively, from cull dairy cows (Dodson and LeJeune 2005). A longitudinal study of *E. coli* O157:H7 in dairy farms in Wisconsin suggested that *E. coli* O157:H7 can persist in a heifer herd for 2 years (Shere et al. 1998). Dairy wastes are the potential sources of a wide variety of zoonotic pathogens that contaminate the environment and water sources and impact water quality (Duffy 2003; Hoar et al. 1999; Lewis et al. 2005; Purdy et al. 2001).

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Antibiotics are used worldwide in dairy operations for the treatment and prevention of clinical disease and for increasing feed efficiency (De Vliegher et al. 2012; Donovan et al. 2002; Watanabe et al. 2008, 2010; Stanton et al. 2010, 2012). As a result, antibiotic-resistant bacteria are found in dairy manure (Esiobu et al. 2002), implicating antibiotics use in dairies as a potential source of antibiotic-resistant and multi-antibiotic-resistant pathogenic bacteria, such as various pathogenic serotypes of *Salmonella enterica* (serotype Newport and serotype Typhimurium). There is the potential of environmental dissemination of and groundwater contamination by antibiotic-resistant bacteria from farm animals.

In rural areas near dairies or other confined animal farming operations, shallow groundwater is the most common source of drinking water in the USA and worldwide (Morris et al. 2003; Kenny et al. 2009). Maintaining good agricultural practices and developing science-based strategies for using antibiotics in dairy operations are of critical importance for protecting not only drinking water resources but also food safety and worker safety. However, such efforts are frequently hampered by a gap of knowledge of bacteria occurrence and antibiotic resistance on dairy operations. The objective of the present work was to determine the occurrence and antibiotic resistance of generic *E. coli* at the ground surface and in groundwater in dairy operations.

## Materials and methods

### Sample collection

Between autumn of 2006 and spring of 2008, four sampling events were conducted to collect samples from two commercial dairies in Modesto County in Northern California. Sources of samples were categorized as (1) calf hutches (water or solids), (2) heifer/cow (pen solid), (3) flush lane or lagoon (slurry), (4) irrigated fields treated with liquid manure (soil), and (5) groundwater well (water). Water samples from calf hutches, slurry samples from flush lanes, and water samples from lagoons were collected by compositing 12 equally sized subsamples into a single sterilized polypropylene bottle. Pen solid and field soil samples were composited from 12 spatially distributed subsamples, collected by using sterilized tongue depressors and composited by mixing and

placing materials into sterilized polypropylene bottles. Depending on the season and site of sampling, pen solids and field soils presented as wet or dry basis. Groundwater was collected from monitoring wells installed at the dairies and screened from 10 to 30 ft below ground surface (BGS) (Harter et al. 2002). Groundwater was directly pumped into sterilized 10-L carboys using a portable and submersible stainless steel Grundfos™ pump. The exterior wall of the hose and pump head were wiped with sterile cloth and the interior of the system (the pump and the tubing) was washed by pumping 50 L of deionized water before use and between wells, a procedure avoiding cross-contamination confirmed by laboratory testing. Samples were maintained on ice during transportation, stored in a cold room (4 °C) upon arrival at the laboratory, and processed for isolating *E. coli* within 24 h after sampling. The number of samples collected from each dairy area during each sampling event is shown in Table 1.

### *E. coli* isolation

For surface samples, approximately 1.0 g pen solids or field soils, 1.0 mL water from calf hutches, or 1.0 mL slurries were suspended in PBS in 50-mL tubes and homogenized by shaking for 15 min using a wrist action shaker. After shaking, solid particulates were removed by filtering through four-layer gauze in a funnel and filtrates were 10-fold serially diluted. Using a vacuum filtering system, prepared samples were filtered through 47-mm diameter 0.45- $\mu$ m pore size nitrocellulose membrane filters. For groundwater samples, a positive pressure vessel filtering system was used to filter 10 L of each sample. Water was filtered through 142-mm diameter 0.45- $\mu$ m pore size nitrocellulose membrane filters. All filters with filtrates were placed onto ChromAgar ECC medium and incubated at 37 °C for 24 h. Two suspected *E. coli* colonies from each plate were used for biochemical tests including triple sugar iron, urea, and Simmons' citrate agar reactions. Numbers of confirmed positive *E. coli* colonies on each plate were counted and concentrations of bacteria were calculated and expressed as numbers of colony-forming units (CFU) per 100 mL of liquid samples or per gram of solid samples. *E. coli* isolates from positive samples were banked and stored at -80 °C until further analysis.

**Table 1** Prevalence and antibiotic resistance of *E. coli* in surface water and groundwater in two dairies in Northern California

| Dairy | Season/year    | Calf hutches (water or soil) |                                 |                         | Heifer/cow (pen solid)  |                                      |                         | Flush lane or lagoon (slurry) |   |                         | Irrigated field (soil)  |                                      |                         | Groundwater (water)     |   |                         |
|-------|----------------|------------------------------|---------------------------------|-------------------------|-------------------------|--------------------------------------|-------------------------|-------------------------------|---|-------------------------|-------------------------|--------------------------------------|-------------------------|-------------------------|---|-------------------------|
|       |                | Positive/ total samples      | <i>E. coli</i> concentration    | (R+IR)/ tested isolates | Positive/ total samples | <i>E. coli</i> concentration (CFU/g) | (R+IR)/ tested isolates | Positive/ total samples       | <i>E. coli</i> concentration (CFU/100 mL) | (R+IR)/ tested isolates | Positive/ total samples | <i>E. coli</i> concentration (CFU/g) | (R+IR)/ tested isolates | Positive/ total samples | <i>E. coli</i> concentration (CFU/100 mL) | (R+IR)/ tested isolates |
| I     | Oct. 2006      | NA                           | NA                              | NA                      | 3/3                     | $2.53 \times 10^6$                   | 0/3                     | 3/3                           | $0.48 \times 10^6$                        | 1/3                     | 1/1                     | 15.3                                 | 1/1                     | 0.01                    | 1/1                                       |                         |
|       | Feb.–Apr. 2007 | 2/2                          | $1.60 \times 10^6$ <sup>a</sup> | 1/2                     | 7/7                     | $1.20 \times 10^6$                   | 2/6                     | 6/6                           | $2.02 \times 10^6$                        | 1/5                     | 0/4                     | 0                                    | 0/2                     | 0                       | NA  |                         |
|       | Sept. 2007     | 2/2                          | $2.95 \times 10^6$ <sup>a</sup> | 1/1                     | 4/4                     | $0.17 \times 10^6$                   | 1/2                     | 4/4                           | $2.83 \times 10^6$                        | 0/1                     | 2/2                     | 260.0                                | 1/2                     | 0.29                    | 2/2                                       |                         |
|       | Feb. 2008      | 1/1                          | $0.08 \times 10^6$ <sup>a</sup> | 1/1                     | 4/4                     | $0.19 \times 10^6$                   | 2/4                     | 2/2                           | $0.91 \times 10^6$                        | 0/2                     | 1/1                     | 420.0                                | 0/1                     | NA                      | 0/1                                       |                         |
|       | Mean           | 5/5                          | $1.54 \times 10^6$ <sup>a</sup> | 3/4                     | 18/18                   | $1.02 \times 10^6$                   | 5/15                    | 15/15                         | $1.56 \times 10^6$                        | 2/11                    | 4/8                     | 173.8                                | 3/4                     | 0.10                    | 2/6                                       |                         |
| II    | Oct. 2006      | 1/1                          | $2.93 \times 10^4$ <sup>b</sup> | 1/1                     | 4/4                     | $2.66 \times 10^6$                   | 2/4                     | 2/2                           | $0.42 \times 10^6$                        | 1/2                     | 2/2                     | 3,256.0                              | 0/2                     | 0                       | 0/2                                       |                         |
|       | Feb.–Apr. 2007 | 2/2                          | $0.52 \times 10^4$ <sup>b</sup> | 1/2                     | 7/7                     | $2.62 \times 10^6$                   | 1/7                     | 6/6                           | $0.32 \times 10^6$                        | 3/6                     | 2/4                     | 6.0                                  | 1/2                     | 352.7                   | 2/2                                       |                         |
|       | Sept. 2007     | 1/1                          | $1.03 \times 10^4$ <sup>b</sup> | 1/1                     | 4/4                     | $2.10 \times 10^6$                   | 1/3                     | 4/4                           | $0.73 \times 10^6$                        | 1/2                     | 4/4                     | 458.8                                | 1/1                     | 1.11                    | 2/2                                       |                         |
|       | Feb. 2008      | 1/1                          | $0.10 \times 10^4$ <sup>b</sup> | 0/1                     | 4/4                     | $2.12 \times 10^6$                   | 2/4                     | 2/2                           | $0.54 \times 10^6$                        | 2/2                     | 2/2                     | 759.5                                | 1/2                     | NA                      | NA  |                         |
|       | Mean           | 5/5                          | $1.15 \times 10^4$              | 3/5                     | 19/19                   | $2.38 \times 10^6$                   | 6/18                    | 14/14                         | $0.50 \times 10^6$                        | 7/12                    | 10/12                   | 1,120.1                              | 4/7                     | 117.9                   | 4/6                                       |                         |
| Total | 10/10          |                              | 6/9                             | 37/37                   |                         |                                      | 11/33                   | 29/29                         |   | 9/23                    | 14/20                   |                                      | 7/11                    | 6/12                    | 1/4                                       |                         |

R resistance, IR intermediate resistance, NA no samples available

<sup>a</sup> CFU/100 mL

<sup>b</sup> CFU/g

## Determining *E. coli* susceptibility to antibiotics

Susceptibility to antibiotics was determined using a minimum inhibitory concentration (MIC) method according to the Clinical and Laboratory Standards Institute (CLSI) (M02-A10; 2009) with minor modifications. Briefly, three to five colonies of overnight-grown fresh *E. coli* were inoculated to 4 mL demineralized water and turbidities of the inoculated suspensions were measured using a spectrophotometer (625 nm) and adjusted to turbidity comparable to that of the 0.5 McFarland turbidity standards. Ten microliters of the suspensions was transferred into a tube containing 11 mL Sensititre Muller–Hinton broth with TES buffer to yield a concentration of  $1 \times 10^5$  CFU/mL. Fifty microliters of the broth suspension was inoculated into each well of custom-made Sensititre® plates—CMV1AGNF (Trek Diagnostic Systems Inc., Westlake, OH, USA). *E. coli* strain ATCC 25922 was used as reference strain for quality control as recommended in the CLSI manual. Plates were incubated at 34–36 °C for 18–24 h and read by using a mini light viewing box. Growth of bacteria appears as turbidity or as sediment of cells at the bottom of a well. The MIC values were recorded as the lowest concentration of antibiotics that inhibits visible growth of bacteria. Antibiotics on the plates were amikacin (AMI), amoxicillin–clavulanic acid (AUG), ampicillin (AMP), cefoxitin (FOX), ceftiofur (TIO), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfisoxazole (FIS), tetracycline (TET), and trimethoprim/sulfamethoxazole (SXT). Depending on availability, interpretation of susceptibility to antibiotics were based on CLSI criteria for human pathogens for cefoxitin, ceftriaxone, ciprofloxacin, and nalidixic acid (CLSI Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement, M100-S20, Vol. 30, No. 1) or criteria for veterinary pathogens for all other antibiotics (CLSI Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition, M31-A3, Vol. 28, No. 8). The criteria for sensitive (S), intermediate resistance (IR), and resistance (R) to antibiotics are shown in Table 2.

## Statistical analysis

The association between source of solids (manure and soil) or water (calf hutches, flush alley, etc.), season of collection, and mean number of antibiotics that *E. coli* strains were resistant to was evaluated using negative binomial regression using the Stata software (version 9, 2005), with herd set as a cluster variable to control for potential lack of independence between *E. coli* strains at the farm level. Significance was set at  $P \leq 0.05$ .

## Results and discussion

The prevalence of *E. coli* in dairy surface water and groundwater is shown in Table 1. Overall, 88.9 % (96 out of 108) samples were positive for *E. coli* with 100 % detection in slurries from lagoon and flush lane, in solids from heifer/cow pens, and in water and solids from calf hutches. Typical concentrations of *E. coli* range from several hundred thousand to over a million CFU per 100 mL of liquid or per gram of solids, with all-season mean concentrations  $>0.5 \times 10^6$  CFU/100 mL (Table 1). These levels of generic *E. coli* are consistent with what we would expect given the very high proportion of fecal material mixed into these samples, sometimes exceeding 50 % on a wet weight basis in pen surface solid samples. A similar high prevalence of *E. coli* (89 to 100 %) and *Enterococcus* (75 to 100 %) has been documented in northeastern US dairies (Pradhan et al. 2009). Despite these high concentrations of *E. coli* in surface samples in the two dairies, concentrations of *E. coli* in liquid manure-treated, irrigated field soils proximal to the dairies were four magnitudes lower, with mean concentrations of 173 and 1,120 CFU/g in the two dairies. Concentrations of *E. coli* in groundwater ranged from 0 to ~350 CFU/100 mL, which was 3- to 7- $\log_{10}$  lower than that in surface samples such as fecal matter, slurry, and lagoon water (Table 1), but with sometime minimal difference to surface concentrations in surface soils of manure-treated fields. In these two dairies, the average water table depth is approximately 10 ft BGS and the soil type is a sandy loam (Harter et al. 2002); thus, the attenuation of *E. coli* between dairy surface water and groundwater is on the order of 0.3- to 0.7- $\log_{10}$  reduction per foot of subsurface water

**Table 2** Range of antibiotics dilution, criteria of resistance, MIC values, and numbers of resistant *E. coli* isolates

| Antibiotic | Range of dilution (µg/mL) | Criteria of resistance (µg/mL) |      |        | Mean (±SD) MIC of <i>E. coli</i> reaction (µg/mL) | No. (%) of R isolates              | No. (%) of IR isolates | Total no. (%) of R or IR isolates |
|------------|---------------------------|--------------------------------|------|--------|---|------------------------------------|------------------------|-----------------------------------|
|            |                           | S                              | IR   | R      |   |                                    |                        |                                   |
| FOX        | 0.5–64                    | ≤8                             | 16   | ≥32    | 16.51 (23.85)                                     | 19 (23.8)                          | 1 (1.3)                | 20 (25.0)                         |
| AMI        | 0.25–128                  | ≤16                            | 32   | ≥64    | 5.24 (19.82)                                      | 2 (2.5)                            | 0 (0)                  | 2 (2.5)                           |
| CHL        | 1–64                      | ≤8                             | 16   | ≥32    | 10.98 (14.84)                                     | 8 (10.0)                           | 2 (2.5)                | 10 (12.5)                         |
| TET        | 2–64                      | ≤4                             | 8    | ≥16    | 14.20 (23.69)                                     | 19 (23.8)                          | 1 (1.3)                | 20 (25.0)                         |
| AXO        | 0.125–128                 | ≤1                             | 2    | ≥4     | 4.79 (20.44)                                      | 11 (13.8)                          | 0 (0)                  | 11 (13.8)                         |
| AUG        | 0.5/0.25–64/32            | ≤8/4                           | 16/8 | ≥32/16 | 12.01 (17.35)/6.02 (8.67)                         | 14 (17.5)                          | 5 (6.3)                | 19 (23.8)                         |
| CIP        | 0.0075–4                  | ≤1                             | 2    | ≥4     | 0.024 (0.11)                                      | 0 (0)                              | 0 (0)                  | 0 (0)                             |
| GEN        | 0.125–32                  | ≤4                             | 8    | ≥16    | 1.75 (6.02)                                       | 3 (3.8)                            | 0 (0)                  | 3 (3.8)                           |
| NAL        | 0.25–32                   | ≤16                            | NA   | ≥32    | 2.88 (3.81)                                       | 1 (1.3)                            | 0 (0)                  | 1 (1.3)                           |
| TIO        | 0.125–16                  | ≤2                             | 4    | ≥8     | 2.08 (4.23)                                       | 12 (15.0)                          | 1 (1.3)                | 13 (16.3)                         |
| FIS        | 8–512                     | ≤256                           | NA   | ≥512   | 102.60 (177.88)                                   | Dilution range out of MIC criteria |                        |                                   |
| SXT        | 0.06/1.19–8/152           | ≤2/38                          | NA   | ≥4/76  | 0.42 (1.57)/8.84 (33.06)                          | 4 (5.0)                            | 0 (0)                  | 4 (5.0)                           |
| KAN        | 4–128                     | ≤16                            | 32   | ≥64    | 15.00 (35.24)                                     | 7 (8.8)                            | 0 (0)                  | 7 (8.8)                           |
| AMP        | 0.5–64                    | ≤8                             | 16   | ≥32    | 12.33 (20.47)                                     | 12 (15.0)                          | 6 (7.5)                | 18 (22.5)                         |
| STR        | 16–128                    | NA                             | NA   | NA     | 24.60 (26.57)                                     | No MIC criteria available          |                        |                                   |

R resistance, IR intermediate resistance, S sensitive, NA not available

transport (less underneath manure-treated fields). These substantive reductions in *E. coli* concentrations within the unsaturated zone above the water table are important for reducing or preventing groundwater contamination with bacteria from surface sources.

Large amounts of genetic diversity exist in populations of generic *E. coli* (Aslam et al. 2004; Dimri et al. 1992; Ibekwe et al. 2011). Therefore, isolates may vary between each other from different categories or even within a category of samples in this work given the size and age groups of animal populations housed within the two operations. Since our objective is to compare overall environmental loads of *E. coli* populations and antibiotic resistance in surface water and in groundwater in dairy operations, this initial work did not determine the genetic relationships between different isolates, especially the similarity between isolates from groundwater and from surface samples. In future work, it will be important to conduct genetic analysis using DNA fingerprinting methods such as PFGE in our ongoing studies.

Eighty of the 96 *E. coli* isolates were successfully retrieved and used for testing susceptibility to antibiotics

(Table 1). Among the 80 *E. coli* isolates tested (35 isolates from dairy I and 45 isolates from dairy II), 34 (42.5 %) isolates exhibited resistance or intermediate resistance to one or more antibiotics (Table 1). *E. coli* was mainly resistant to the antibiotics tetracycline (25.0 %), cefoxitin (25.0 %), amoxicillin/clavulanic acid (23.8 %), and ampicillin (22.5 %) (Table 2). Eleven isolates from each dairy (27.5 % of all isolates) were found to be multi-antibiotic resistant (resistant to ≥3 antibiotics). Importantly, *E. coli* isolated from water or soils around the calf hutch area was resistant to significantly larger numbers of antibiotics compared to strains of *E. coli* isolated from heifer/cow pen solids or from slurry and water in the flush alley lanes and manure storage lagoon (Table 3). Previous work on dairies in San Joaquin Valley, California found widespread occurrence of resistance to antibiotics among *E. coli* isolated from calf feces (Berge et al. 2005). In these work, similar patterns of antibiotic resistance in *E. coli* from the calf hutch area was observed. In this study, solid samples from the calf hutch areas were from areas that had been dedicated for many years to calf raising. In contrast, heifer/cow pens in this study included a variety

**Table 3** Negative binomial regression model of the association between source of sample and season of sampling on the risk of *E. coli* being resistant to an increasing number of antibiotics

| Risk factor                               | Unadjusted no. of antibiotics <i>E. coli</i> is resistant to <sup>a</sup> | Coefficient | <i>P</i> value <sup>b</sup> | 95 % CI <sup>b</sup> |
|---|---|-------------|-----------------------------|----------------------|
| Sample source                             |   |             |                             |                      |
| Calf hutches (water or soil) <sup>c</sup> | 3.3   | –           |                             |                      |
| Heifer/cow (pen solid)                    | 1.5   | –1.21       | 0.008                       | (–2.1, –0.3)         |
| Flush lane or lagoon (slurry)             | 0.3   | –2.41       | <0.001                      | (–3.3, –1.5)         |
| Irrigated field (soil)                    | 1.4   | –0.77       | 0.06                        | (–1.6, 0.02)         |
| Groundwater well (water)                  | 0.5   | –1.78       | 0.32                        | (–5.3, 1.7)          |
| Season                                    |   |             |                             |                      |
| Feb. <sup>c</sup>                         | 2.2   | –           |                             |                      |
| April                                     | 0.7   | –1.32       | 0.02                        | (–2.4, –0.2)         |
| Sept.–Oct.                                | 1.3   | –0.81       | <0.001                      | (–1.1, –0.5)         |
| Constant for the model                    | –   | 2.01        | <0.001                      | (1.6, 2.4)           |

Resistance to antibiotics determined by MIC that equaled or exceeded fully resistant status as determined by CLSI. Each isolate of *E. coli* could be resistant to up to 15 antibiotics

<sup>a</sup> Crude or unadjusted mean number of antibiotics that *E. coli* strains were resistant to (i.e., source and season not adjusted for each the other) (per gram solids or 100 mL liquids)

<sup>b</sup> Adjusted for potential lack of independence for isolates of *E. coli* cultured from the same dairy

<sup>c</sup> Referent condition for the negative binomial regression model

of dairy locations where animals of different age groups had access at various times of the year. It appears likely that high resistance was related to the calves, while high resistance associated with other age groups may have been masked. What remain unclear is whether ambient conditions and desiccation of manure will influence the occurrence and composition of antibiotic resistance of *E. coli* relative to fresh fecal samples. Among the four groundwater isolates of *E. coli*, one isolate *E. coli* (25 %) exhibited resistance to ceftriaxone and tetracycline and intermediate resistance to chloramphenicol. Although this was only a single isolate among our group of groundwater isolates, the result indicate the potential risk of groundwater contamination with antibiotic-resistant bacteria from dairy farm operations. Several field studies have documented that swine farm operations distribute antibiotic-resistant bacteria to groundwater (Anderson and Sobsey 2006; Chee-Sanford et al. 2001; Koike et al. 2007; Mackie et al. 2006; Sapkota et al. 2007). A laboratory study using column experiments has reported that tetracycline-resistant *Burkholderia cepacia* can transport in porous media (Rysz and Alvarez 2006). Clearly, further studies are warranted to better understand the dynamics of underground transportation of antibiotic-resistant bacteria and develop on-farm interventions to prevent groundwater contamination.

As shown in Table 3, strains of *E. coli* isolated during February were resistant to significantly larger numbers of antibiotics compared to strains of *E. coli* isolated during spring or fall. The coefficients in the negative binomial regression model provide an estimate of the mean number of antibiotics that isolates of *E. coli* were resistant to, while the analysis controls for both source of isolate and season. For example, *E. coli* isolated from a heifer pen during April were resistant to an average of 0.6 antibiotics ( $e^{(2.01-1.21-1.32)}=0.6$ ). In contrast, *E. coli* isolated from soil underneath calf hutches during February (i.e., the referent condition) were resistant to an average of 7.5 of the 15 antibiotics that were examined ( $e^{(2.01)}=7.5$ ). Higher occurrence of antibiotic resistance in February may be associated with cool and wet winter weather conditions and a seasonal increase in antibiotics used in these dairy operations.

According to a survey conducted by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture, approximately 60 % of dairy operations in the USA use antibiotics to treat pre-weaned heifers for disease, primarily respiratory disorders and diarrhea or other digestive problems (Retrieved Info Sheet from APHIS). Sulfonamide and tetracycline are the most common antibiotics used to treat

pre-weaned heifers. In the present work, we found that *E. coli* was mainly resistant to tetracycline (25.0 %), cefoxitin (25.0 %), amoxicillin/clavulanic acid (23.8 %), and ampicillin (22.5 %), which was consistent with the wide use of these agents in dairy operations (Watanabe et al. 2010). In contrast, bacteria were less resistant to amikacin (2.5 %), gentamicin (3.8 %), nalidixic acid (1.3 %), kanamycin (5.0 %), and ciprofloxacin (0 %), which were minimally used on these dairies (Table 2; Watanabe et al. 2010).

Ceftiofur-resistant *E. coli* has been documented in dairy calves (Donaldson et al. 2006). Phenotypes of antibiotic resistance of *E. coli* in dairy farms present in the present work not only implies the importance to animal health and water and environment quality but also the potential impact on human health if the antibiotic-resistant gene transmitted to human flora through water, food, or via aerial pathways (dust) or direct human contact. For example, the resistance to cefoxitin and ceftiofur indicates the presence of enzymes including extended-spectrum beta-lactamase and cephalosporinase produced by corresponding resistant bacteria. The activity spectrum of cefoxitin and ceftiofur includes a broad range of gram-negative and gram-positive bacteria. Resistance to these antibiotics can cause significant problems for the treatment of human infections in hospitals. *E. coli* is a common indicator organism for the presence of fecal pathogens. Data from our present work indicate that similar patterns of antibiotic resistance may be present in pathogenic bacteria that exist in dairy operations and potentially migrate into groundwater.

In conclusion, generic *E. coli* was ubiquitous in surface liquids and solids across two typical dairy operations, yet groundwater under the influence of these high surface microbial loadings had substantially fewer bacteria (3- to 7- $\log_{10}$  reduction). Antibiotic resistance was prevalent among *E. coli* found within the various areas of the dairy operation, especially in the calf hutch areas. Our work indicates that there is also a potential risk of transport of antibiotic-resistant bacteria from dairy surface water to groundwater.

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