Clinical Microbiology: Open Access

Li et al., Clin Microbiol 2018, 7:2
DOI: 10.4172/2327-5073.1000311

Research Article Open Access

Phenotypic Antimicrobial Resistance Profiles of *E. coli* and *Enterococcus* from Dairy Cattle in Different Management Units on a Central California Dairy

Xunde Li[1,2*], Sharif S. Aly[2,3], Zhengchang Su[4], Richard V. Pereira[2], Deniece R. Williams[3], Paul Rossitto[3], John D. Champagne[3], Jennifer Chase[1], Tran Nguyen[1] and Edward R. Atwill[1,2*]

[1]*Western Institute for Food Safety and Security, University of California, Davis, USA*

[2]*Department of Population Health and Reproduction, University of California, Davis, USA*

[3]*Veterinary Medicine Teaching and Research Centre, University of California, Davis, USA*

[4]*College of Computing and Informatics, University of North Carolina Charlotte, USA*

[*]***Corresponding authors:*** *Xunde Li, Ph.D, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, 4213 Vet Med 3B, Davis, CA 95616, USA, Tel: 530-754-9752; E-mail:* xdli@ucdavis.edu

Edward R. Atwill, Ph.D, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, 4207 Vet Med 3B, Davis, CA 95616, USA, Tel: 530-754-2154 E-mail: ratwill@ucdavis.edu

Received date: April 5, 2018; **Accepted date:** April 10, 2018; **Published date:** April 20, 2018

Abstract

The objective of the study was to characterize profiles of phenotypic antimicrobial resistance of enteric bacteria from dairy cattle in different ages and management units. Feces were collected from Holstein and Jersey cattle in different management units on a large Central California dairy including hutch calves (pre-weaned), post-weaned heifers, breeding heifers, springer's (pregnant nulliparous females due to calve), fresh (recently calved) uniparous (first lactation) cows, fresh multiparous (second or greater lactation) cows, mid-lactation multiparous cows, pregnant late lactation multiparous cows, far-off (recently) dry cows (non-lactating), close up (1-3 week due to calve) dry cows, hospital pen, and mid to late lactation multiparous cows. *E. coli* and *Enterococcus* were isolated from fecal samples from cattle in different management units and tested for susceptibility to antimicrobial drugs. *E. coli* from hutch calves showed a wide spectrum of resistance to antimicrobial drugs compared to isolates from other management units. *Enterococcus* isolated from all management units was resistant to a wide spectrum of antimicrobial drugs. The drugs that *E. coli* and *Enterococcus* were most likely resistant to were tetracycline and lincomycin, respectively. Results of this cross-sectional study showed different antimicrobial resistance profiles of bacteria from dairy cattle of different ages and in different management units. Information can be considered for farm managements to mitigate antimicrobial resistance.

Keywords: Antimicrobial resistance; Cattle; *E. coli*; *Enterococci*; Dairy

Introduction

Despite the increasing concerns of food-producing animal agriculture as a major contributor to biological reservoirs of antimicrobial resistance genes that disseminate antimicrobial resistance in the environment [1-5], the status of antimicrobial resistance in animal agriculture has not been fully characterized due to challenges such as the complexity and diversity of livestock production systems, diverse bacteria communities on farms, different drug use practices, and diverse farm management systems. Dairy farms are composed of multiple inter-connected management units that are designed for differences in age, nutritional needs, production, and reproductive status that may differ depending on facility size and design, farm management, cattle's age or parity. Commonly these management units include hutch calves (pre-weaned), post-weaned heifers, breeding heifers, springer's (pregnant nulliparous females due to calve within 1-4 weeks), fresh (recently calved) uniparous and multiparous cows, mid to late lactation uniparous cows, mid-lactation and pregnant late lactation multiparous cows, far-off dry cows (recently non-lactating), close up dry cows (1-3 week due to calve) and hospital animals. Cattle in such management units may differ in their susceptibility to different diseases and treatment of disease with antimicrobial drugs, which in turn result in different conditions for acquisition and transmission of antimicrobial resistance (e.g. high selective pressures and high concentrations of relevant enteric bacteria can result in high probability for selection, survival, and transfer of resistance genes) [6,7]. Such an on-farm network of management units could select for reservoirs of bacteria with different resistance gene composition and intensity of gene expression, hence, different antimicrobial resistance phenotypic profiles. The patterns of antimicrobial resistance and their ecological connectivity of bacteria among animals in these different management units remain poorly understood. Although there are numerous publications on antimicrobial resistance on dairies [6,8-10], many studies have been based on animals in selected management units or production stages. As a result, both the probability (low, medium, high) of antimicrobial resistance and the spectrum of resistance (narrowly *vs.* broadly) in all management units on dairies remain poorly characterized, which hampers our ability to mitigate antimicrobial resistance in dairy production settings. In this work, our hypothesis was that the proportion and spectrum of antimicrobial resistance in generic *E. coli* and *Enterococcus* differ between cattle in different management units on a dairy. Our objective was to conduct a cross-sectional study of the

phenotypic traits of antimicrobial resistance of generic *E. coli* (gram-negative) and *Enterococcus* (gram-positive) from a convenience sample of cattle in different management units on a typical large commercial California dairy.

Materials and Methods

Study herd

A dairy herd was identified based on willingness of the herd veterinarian and management to participate in an antimicrobial resistance study. The study dairy was in the San Joaquin Valley milk shed where the majority of California's dairy operations are located and over 85% of state's milk is produced. The study dairy consisted of approximately 3,700 milking cows with 64% Jersey and 36% Holstein cows. Lactating dairy cattle were housed in free stall barns bedded with dried manure solids. Young stock, dry cows and hospital cows were housed in open-lot pens with concrete flush lanes in the feed alley. Hutch calves were housed in elevated "California-style" wooden hutches over a flush lane. All flush lanes used recycled lagoon water for flushing.

Collection of fecal samples

On a single day in June 2016, fecal samples were collected from management units identified in Table 1. Cattle in each of these management units were identified based on convenience sampling. Trained study personnel collected the fecal samples manually from the rectum of cattle using individual disposable sleeves.

Management Unit	Estimated no. of cattle in sampled pen	Population size of management unit	No. samples from sampled pen	Description of management unit	Estimated duration of housing in management unit
Hutch calves	770	770	9	Individually-housed calves from birth to approximately 1-2 weeks after weaning	70 days
Post weaned heifers	100	2000	6	Group-housed heifers and bull calves fed solid diet	6 to 12 months
Breeding heifers	170	600	8	Heifers at breeding height and age, approximately 13 to 15 months old	1 to 3 months
Springers	75	120	8	Pregnant nulliparous heifers within 1 to 4 weeks of calving	1 to 4 weeks
Fresh uniparous cows	50	185	8	First lactation cows within 1 to 2 months post-calving	4 to 8 weeks
Fresh multiparous cows	90	400	8	Second lactation or greater cows within 1 to 2 months post-calving	4 to 8 weeks
Mid lactation uniparous cows	690	865	8	First lactation cows 60 to 250 Days in Milk	6 months
Mid-lactation multiparous cows	206	1330	8	Second lactation or greater cows 60 to 250 Days in Milk	6 months
Pregnant late lactation multiparous cows	420	895	8	Second lactation or greater cows >250 Days in Milk	2 to 8 months
Far-off cows	250	280	3	Multiparous dry cows 21 to 60 days prior to calving	30 to 40 days
Close up cows	110	175	8	Multiparous dry cows within 21 prior to calving	21 to 30 days
Hospital	40	40	8	Lactating cows treated with medication that requires milk withdrawal	3 to 14 days

Table 1: Management units on a large California dairy, 2016.

Fecal samples were then transferred into polypropylene tubes. Samples were shipped on ice on the day of collection to UC Davis and stored at 4°C before processing within 24 h of collection. Sampling was approved by the Institutional Animal Care and Use Committee (IACUC) of University of California Davis (protocol number 18941).

Isolation *E. coli* and *Enterococcus* from feces

E. coli and *Enterococcus* were isolated from fecal samples using previously described methods [11] with modifications. Briefly, 5 g of feces was dispersed in 40 mL of Buffered Peptone Water (BPW) in 50 mL tubes and homogenized by shaking for 15 min using a mechanical shaker. After shaking, solid particulates were removed by filtering through four-layer gauze in a funnel sited on a new 50 mL tube. For isolation of *E. coli* and *Enterococcus*, 1000, 100, and 10 µl of the fecal solutions were streaked onto CHROMAgar ECC and *Enterococcus* Indoxyl-β-D-Glucoside agar (mEI) plates, respectively. CHROMagar ECC plates were incubated at 37°C for 24 h and mEI plates were incubated at 41°C for 48 h. *E. coli* (ATCC 25922), and *Enterococcus faecalis* (ATCC 29212) on agar plates were used as positive controls. After incubation, at least three presumptive positive colonies from each type of agar plate were used for confirmation using biochemical

tests. Generic *E. coli* was confirmed by biochemical tests including Indole, triple sugar iron, Urea, and Simmons Citrate, and Methyl Red–Voges–Proskauer; *Enterococcus* was confirmed by biochemical tests including Brain Heart Infusion agar, Brain Heart Infusion Broth, Brain Heart Infusion Broth with 6.5% NaCl, and Bile Esculin reactions. Confirmed colonies of each bacteria species were streaked onto the same agar plates again and incubated in the same conditions as above to isolate pure colonies. Two pure colonies of each bacterium from each positive sample were stored in cryovial at -80°C for further analysis.

Assay of bacterial susceptibility to antimicrobial drugs

Bacterial susceptibility to antimicrobial drugs was tested using a minimum inhibitory concentration (MIC) method as previously described [12] with minor modifications. Briefly, three to five colonies of a pure culture of *E. coli* or *Enterococcus* was inoculated into 4 mL of demineralized water. Turbidity's of bacterial solutions 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 bacterial solutions were transferred into a tube containing 11 mL Sensititre Muller-Hinton broth with TES buffer to yield a concentration of 1×10^5 CFU/mL. Fifty microliters of *E. coli* solutions was inoculated into each well of gram-negative (G-) plates and *Enterococcus* solutions was inoculated into each well of gram-positive (G+) plates (Trek Diagnostic Systems Inc., Westlake, OH). Reference strain of *E. coli* (ATCC 25922) was used as quality control in the G- plates while *Enterococcus faecalis* (ATCC 29212) was used as quality control in the G+ plates. After incubation at 36°C for 24 h, plates were read using a mini light viewing box and 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 concentrations of antimicrobial drugs that inhibit visible growth of bacteria. Antimicrobial drugs on the G- plates were cefoxitin (FOX), azithromycin (AZI), chloramphenicol (CHL), tetracycline (TET), ceftriaxone (AXO), amoxicillin/clavulanic acid (AUG), ciprofloxacin (CIP), gentamycin (GEN), nalidixic acid (NAL), ceftiofur (XNL), sulfisoxazole (FIS), trimethoprim-sulfamethoxazole (SXT), ampicillin (AMP), and streptomycin (STR). Antimicrobial drugs on the G+ plates were tigecycline (TGC), tetracycline (TET), chloramphenicol (CHL), daptomycin (DAP), streptomycin (STR), tylosin tartrate (TYLT), quinupristin/dalfopristin (SYN), linezolid (LZD), nitrofurantoin (NIT), penicillin (PEN), kanamycin (KAN), erythromycin (ERY), ciprofloxacin (CIP), vancomycin (VAN), lincomycin (LIN), and gentamycin (GEN). Interpretations of bacterial susceptibility to antimicrobial drugs were based on the criteria of resistant (R), intermediate resistant (IR), and susceptible (S) for bacteria isolated from animals established by the Clinical and Laboratory Standard Institute (CLSI) [13].

Results and Discussion

Management units	No. of animals sampled	No. of animals tested for	No. of *E. coli* isolates resistant to tested antimicrobial drugs													
			FOX	AXO	AUG	AMP	XNL	AZI	CHL	TET	CIP	NAL	FIS	SXT	GEN	STR
Hutch calves	9	5	1	2	1	2	1	0	4	5	1	2	0	2	2	3
Post wean heifers	6	6	0	0	0	0	0	0	0	3	0	0	0	1	0	1
Breeding heifers	8	5	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Springers	8	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fresh first lactation cows	8	5	1	1	0	0	0	0	2	1	0	0	0	2	0	1
Fresh second or greater lactation cows	8	5	0	0	0	0	0	0	1	1	0	0	0	1	0	1
Mid-late lactation first lactation cows	8	5	0	1	0	0	1	0	0	0	1	0	0	1	1	0
Mid-lactation second or greater lactation cows	8	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Preg late lactation cows	8	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Far-off cows	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Close up cows	8	6	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Hospital	8	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0
E. coli (control)	-	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1
Pseudomonus (control)	-	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0

[a] Numbers include both resistant (R) and intermediate resistant (IR) isolates.

Table 2: Antimicrobial resistance of fecal *E. coli* isolated from a convenience sample of cattle in different management units on a large California dairy, 2016[a].

In total 90 animals were sampled from 12 different management units on the dairy farm. Numbers of animals sampled from each management unit are shown in Tables 1-3. All 90 fecal samples from individual animals tested positive for both *E. coli* and *Enterococcus*. Due to limited funding, we selected a random sample of 59 isolates of *E. coli* and 59 isolates of *Enterococcus* from individual animals covering the 12 management units to test their susceptibility of antimicrobial drugs (Tables 2 and 3).

Management units	No. of animals sampled	No. of animals tested for *Enterococcus* susceptibility	No. of *Enterococcus* isolates resistant to tested antimicrobial drugs														
			TGC	TET	CHL	DAP	TYLT	ERY	SYN	LZD	NIT	PEN	CIP	VAN	LIN	KAN	STR	GEN
Hutch calves	9	5	0	4	3	0	4	4	4	3	3	0	5	0	5	4	3	1
Post wean heifers	6	6	0	2	2	0	1	3	0	2	5	0	5	0	4	2	1	1
Breeding heifers	8	5	0	2	1	0	2	3	1	5	3	0	4	1	3	5	0	2
Springers	8	5	0	2	2	0	3	2	2	4	5	1	3	1	5	4	1	3
Fresh heifers	8	5	0	0	2	0	1	5	2	3	2	0	5	0	3	3	0	0
Fresh cows	8	5	0	1	1	0	1	1	0	2	2	0	4	1	4	3	0	1
Mid-late lactation heifers	8	5	0	1	2	0	2	3	4	5	1	0	4	0	5	4	0	0
Mid-lactation cows	8	4	0	2	2	0	1	3	3	3	1	0	4	0	4	1	0	1
Preg late lactation cows	8	5	0	0	2	0	2	4	2	4	4	0	5	1	5	3	1	2
Far-off cows	3	3	0	1	1	0	1	2	0	1	2	0	3	1	2	1	0	1
Close up cows	8	6	0	1	1	0	2	3	2	2	3	0	6	1	4	2	0	2
Hospital	8	5	0	2	1	0	3	5	3	5	3	1	5	1	5	5	1	1
Enterococcus (control)	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. pyogen (control)	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Numbers include both resistant (R) and intermediate resistant (IR) isolates

Table 3: Antimicrobial resistance of fecal *Enterococcus* from a convenience sample of cattle at different management units on a large California dairy, 2016[a]

Overall, different spectrums of resistance to tested antimicrobial drugs were observed between *E. coli* and *Enterococcus*. Among antimicrobial drugs tested, *E. coli* was mostly resistant to tetracycline (22% isolates), trimethoprim-sulfamethoxazole (14% isolates), and streptomycin (10% isolates) (Table 4). *Enterococcus* was mostly resistant to lincomycin (76% isolates), ciprofloxacin (66% isolates), and kanamycin (66% isolates) (Table 4).

E. coli isolates collected from hutch calves showed a wider spectrum of resistance to tested antimicrobial drugs compared to bacteria isolates from other management units. For instance, among the five *E. coli* isolates from the calves, five were resistant to tetracycline, four were resistant to chloramphenicol, three were resistant to streptomycin, and two were resistant to ceftriaxone, gentamycin, nalidixic acid, trimethoprim-sulfamethoxazole, and ampicillin (Table 2). For *Enterococcus*, isolates from animals in all management units were resistant to a wide spectrum of tested antimicrobial drugs (Table 3).

Different phenotypic traits of single and multidrug resistance were observed between *E. coli* and *Enterococcus* isolates. For *E. coli*, all isolates from hutch calves were 100% resistant to multiple drugs (MDR) but the prevalence of MDR was less than 100% from animals in other managements units. For, *Enterococcus* 83% of isolates from the post weaned heifers were resistant to ≥ 1 drug and 33% were resistant to ≥ 3 drugs; *Enterococcus* isolates from all other management units were 100% resistant to ≥ 1 drug and over 50% isolates were resistant to ≥ 3 drugs. Despite the small sample size, results of the present study demonstrate the diverse patterns of antimicrobial resistance of commensal bacteria from animals in different management units of the entire farm at the time samples were collected. Observed higher prevalence of antimicrobial resistance of *E. coli* and *Enterococcus* from hutch calves was in agreement with the results from another study where prevalence of antimicrobial resistant bacteria was highest in younger animals [14].

Bacteria	Class of Antimicrobials	Drugs	No. (%) of susceptible (S) isolates	No. (%) of intermediate resistant (IR) isolates	No. (%) of resistant (R) isolates
E. coli	Aminoglycosides	Gentamycin	56 (94.9)	1 (1.7)	2 (3.4)
		Streptomycin	53 (89.8)	0 (0)	6 (10.2)
	Beta-lactam	Amoxicillin/clavulanic acid	58 (98.3)	1 (1.7)	0 (0)
		Ampicillin	56 (94.9)	0 (0)	3 (5.1)
		Cefoxitin	57 (96.6)	0 (0)	2 (3.4)
		Ceftiofur	56 (94.90	1 (1.7)	2 (3.4)
		Ceftriaxone	55 (93.2)	1 (1.7)	3 (5.1)
	Chloramphenicol	Chloramphenicol	52 (88.1)	2 (3.4)	5 (8.5)
	Macrolides	Azithromycin	59 (100)	0 (0)	0 (0)
	Quinolones	Ciprofloxacin	57 (96.6)	0 (0)	2 (3.4)
		Nalidixic acid	57 (96.6)	0 (0)	2 (3.4)
	Sulfonamides	Sulfisoxazole	59 (100)	0 (0)	0 (0)
		Trimethoprim-sulfamethoxazole	51 (86.4)	0 (0)	8 (13.6)
	Tetracycline	Tetracycline	46 (78.0)	0 (0)	13 (22.0)
Enterococcus	Aminoglycosides	Gentamycin	44 (74.6)	0 (0)	15 (25.4)
		Kanamycin	22 (37.3)	0 (0)	37 (62.7)
		Streptomycin	52 (88.1)	0 (0)	7 (11.9)
	Beta-lactam	Penicillin	57 (96.6)	0 (0)	2 (3.4)
	Chloramphenicol	Chloramphenicol	39 (66.1)	9 (15.3)	11 (18.6)
	Glycopeptides	Vancomycin	52 (88.1)	4 (6.8)	3 (5.1)
	Glycylcycline	Tigecycline	47 (79.7)	ND[a]	ND[a]
	Lincosamides	Lincomycin	10 (16.9)	4 (6.8)	45 (76.3)
	Lipopeptides	Daptomycin	44 (74.6)	ND[a]	ND[a]
	Macrolides	Erythromycin	21 (35.6)	21 (35.6)	17 (28.8)
		Tylosin tartrate	36 (61.0)	1 (1.7)	22 (37.3)
	Nitrofurans	Nitrofurantoin	25 (42.4)	34 (57.6)	0 (0)
	Oxazolidinones	Linezolid	20 (33.9)	10 (16.9)	29 (49.2)
	Quinolones	Ciprofloxacin	6 (10.2)	14 (23.7)	39 (66.1)
	Streptogramins	Quinupristin/dalfopristin	36 (61.0)	4 (6.8)	19 (32.2)
	Tetracycline	Tetracycline	41 (69.5)	1 (1.7)	17 (28.8)

[a] Not determined due to the MIC breakpoints outranged the CLSI criteria

Table 4: Overall antimicrobial resistance of *E. coli* and *Enterococcus* from cattle in different management units in a large dairy in Central California, 2016.

Such a difference in occurrence of antimicrobial resistance could be due to calves' higher susceptibility to respiratory and diarrhea diseases which require more intensive use of antimicrobial drugs to prevent and treat such diseases compared to cattle in other management units/age groups. For example, Pereira et al. reported that treatment of preweaned dairy calves with ceftiofur resulted in a higher prevalence of isolates resistant to ≥ 3 antimicrobial drugs (97%) compared calves with no treatment with ceftiofur (73%) [6]. However, the prevalence of

resistant bacteria is not necessarily always related to recent use of antimicrobial drugs, instead, neonate-adapted antimicrobial resistant bacteria could be responsible for the high prevalence of resistant bacteria in calves too [14]. In addition, the greater fitness advantage of antimicrobial resistant bacteria in calves could also be related to the farm environment and the diet exerting selective pressures responsible for the maintenance of antimicrobial resistance genes [15]. Although we did not collect information on management practices including drug uses in different management units, data from this cross-sectional study indicate that improved management of higher risk groups (e.g. hutch calves) can potentially reduce the spread and persistence of antimicrobial resistance on farms.

Communities of enteric bacteria in dairy cattle are composed of a large diversity of commensal, pathogenic and opportunistic bacteria including gram-negative (G-) and gram-positive (G+) bacteria [16]. Exchange and transfer of antimicrobial resistance genes likely occurs more frequently between related organisms [17] which can lead to the formation of hubs of genetically related bacteria sharing similar resistance genes and phenotypic resistance traits. It is impractical to characterize phenotypic antimicrobial resistance profiles of the entire communities of bacteria in dairy cattle at different managements units using culture based methods. Therefore, using the ubiquitous generic *E. coli* (G-) and *Enterococcus* (G+) as surrogates, this study provides an overall profile about the phenotypic traits of antimicrobial resistance of G- and G+ bacterial communities from different management units on dairy farms. Although we did not study bacteria communities involved in the resistance, different resistance patterns between *E. coli* and *Enterococcus* from dairy cattle in different management units/age groups were observed in this cross-sectional study, probably due to different mechanisms of resistance to antimicrobial drugs between G- and G+ bacteria [16]. All *Enterococcus* isolates were found resistant to at least one of the tested antimicrobial drugs. This is in agreement with our previous observations of the widespread occurrence of antimicrobial resistance in *Enterococcus* in dairy farms in the Central Valley of California [12]. This wide spectrum of resistance could be acquired from the accumulation of resistance associated with the use of antimicrobial drugs [18] in this concentrated and mixed agricultural production region. On the other hand, *Enterococcus* has been found intrinsically resistant to many antimicrobial agents [19,20]. The combination of acquired and intrinsic resistance mechanisms can contribute to the wide spectrum of resistance of *Enterococcus* from all management units (i.e. 100% isolates were resistant to ≥ 1 drug).

The current cross sectional study was conducted on a single dairy farm in an agricultural region that is dominated by dairy production systems. Limitations of this work include the lack of random sampling, small sample size, and the lack of data on management practices and use of antimicrobial drugs, etc. Nevertheless, this study identified antimicrobial resistance patterns including single and multiple drug (resistant to drugs from ≥ 3 classes) resistance of *E. coli* and *Enterococcus* with differential occurrence between different management units on a typical dairy farm in this region. Although we did not survey the use of antimicrobial drugs in this farm, results of this work provide information that could be used to guide future studies to investigate factors that may facilitate the spread of antimicrobial resistance on farms. Amongst the hypotheses that should be tested in future research are the role of improved sanitation of preweaned calf hutches and hospitals pens to reduce environmental dissemination of drug resistant bacteria within the farm and the role of targeted use of antimicrobial drugs to better match bacterial groups (G-, G+) and susceptibility profiles.

Conclusion

Different profiles of phenotypic antimicrobial resistance exist between *E. coli* and *Enterococcus* and among different management units on dairy farms. Improved management of higher risk groups (e.g. hutch calves) can potentially reduce the spread and persistence of antimicrobial resistance on farms. Future studies are warranted to characterize bacterial communities and their resistance genes of multidrug resistance strains from dairy cattle at different management units and potential interventions at the management level that may reduce the spread of antimicrobial resistance.

Acknowledgement

The authors thank the study dairy's management for their participation. The authors thank Dr. Hillary Feldmann and Ms. Ariana Villarreal for their assistance in sample collection. The current research was partially supported by the USDA NIFA Exploratory Research program (2015-67030-23892) and the UC Davis Center for Food Animal Health-Dairy Herd Health & Food Safety Funds (CALV-DHHFS-0053).

Disclosure Statement

No competing financial interests exist.

References

1. Kummerer K (2003) Significance of antibiotics in the environment. J Antimicrob Chemother 52: 5-7.
2. Ward MJ, Gibbons CL, McAdam PR, van Bunnik BA, Girvan EK, et al. (2014) Time-Scaled Evolutionary Analysis of the Transmission and Antibiotic Resistance Dynamics of Staphylococcus aureus Clonal Complex 398. Appl Environ Microbiol 80: 7275-7282.
3. Wieler LH (2014) "One Health"--linking human, animal and environmental health. Int J Med Microbiol 304: 775-776.
4. Gilchrist MJ, Greko C, Wallinga DB, Beran GW, Riley DG, et al. (2007) The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. Environ Health Perspect 115: 313-316.
5. Marshall BM, Levy SB (2011) Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24: 718-733.
6. Pereira RV, Siler JD, Ng JC, Davis MA, Grohn YT, et al. (2014) Effect of on-farm use of antimicrobial drugs on resistance in fecal Escherichia coli of preweaned dairy calves. J Dairy Sci 97: 7644-7654.
7. Pereira RV, Siler JD, Cummings KJ, Davis MA, Warnick LD (2015) Effect of heifer-raising practices on E. coli antimicrobial resistance and Salmonella prevalence in heifer raisers. Epidemiol Infect 143: 3256-3265.
8. Edrington TS, Farrow RL, Hume ME, Anderson PN, Hagevoort GR, et al. (2013) Evaluation of the potential antimicrobial resistance transfer from a multi-drug resistant Escherichia coli to Salmonella in dairy calves. Curr Microbiol 66: 132-137.
9. Gibbons JF, Boland F, Buckley JF, Butler F, Egan J, et al. (2014) Patterns of antimicrobial resistance in pathogenic Escherichia coli isolates from cases of calf enteritis during the spring-calving season. Vet Microbiol 170: 73-80.
10. Duse A, Waller KP, Emanuelson U, Unnerstad HE, Persson Y, et al. (2015) Risk factors for antimicrobial resistance in fecal Escherichia coli from preweaned dairy calves. J Dairy Sci 98: 500-516.

11. Li X, Watanabe N, Xiao C, Harter T, McCowan B, et al. (2014) Antibiotic-resistant E. coli in surface water and groundwater in dairy operations in Northern California. Environ Monit Assess 186: 1253-1260.
12. Li X, Atwill ER, Antaki E, Applegate O, Bergamaschi B, et al. (2015) Fecal indicator and pathogenic bacteria and their antibiotic resistance in alluvial groundwater of an irrigated agricultural region with dairies. J Environ Qual 44: 1435-1447.
13. Watts JL, Shryock TR, Apley MA, Bade DJ, Brown SD, et al. (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard-Third Edition. CLSI 28.
14. Khachatryan AR, Hancock DD, Besser TE, Call DR (2004) Role of calf-adapted Escherichia coli in maintenance of antimicrobial drug resistance in dairy calves. Appl Environ Microbiol 70: 752-757.
15. Khachatryan AR, Hancock DD, Besser TE, Call DR (2006) Antimicrobial drug resistance genes do not convey a secondary fitness advantage to calf-adapted Escherichia coli. Appl Environ Microbiol 72: 443-448.
16. Beceiro AM, Tomas M, Bou G (2013) Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev 26: 185-230.
17. Andam CP, Fournier GP, Gogarten JP (2011) Multilevel populations and the evolution of antibiotic resistance through horizontal gene transfer. FEMS Microbiol Rev 35: 756-767.
18. Hollenbeck BL, Rice LB (2012) Intrinsic and acquired resistance mechanisms in enterococcus. Virulence 3: 421-433.
19. Kristich CJ, Rice LB, Arias CA (2014) Enterococcal Infection-Treatment and Antibiotic Resistance. Massachusetts Eye and Ear Infirmary.
20. Johnston LM, Jaykus LA (2004) Antimicrobial resistance of Enterococcus species isolated from produce. Appl Environ Microbiol 70: 3133-3137.