Invited review: Fate of antibiotic residues, antibiotic-resistant bacteria, and antibiotic resistance genes in US dairy manure management systems

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ABSTRACT

United States dairy operations use antibiotics (primarily β -lactams and tetracyclines) to manage bacterial diseases in dairy cattle. Antibiotic residues, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARG) can be found in dairy manure and may contribute to the spread of antibiotic resistance (AR). Although β -lactam residues are rarely detected in dairy manure, tetracycline residues are common and perhaps persistent. Generally, <15% of bacterial pathogen dairy manure isolates are ARB, although resistance to some antibiotics (e.g., tetracycline) can be higher. Based on available data, the prevalence of medically important ARB on dairy operations is generally static or may be declining for antibiotic-resistant *Staphylococcus* spp. Over 60 ARG can be found in dairy manure (including β -lactam and tetracycline resistance genes), although correlations with antibiotic usage, residues, and ARB have been inconsistent, possibly because of sampling and analytical limitations. Manure treatment systems have not been specifically designed to mitigate AR, though certain treatments have some capacity to do so. Generally, well-managed aerobic compost treatments reaching higher peak temperatures (>60°C) are more effective at mitigating antibiotic residues than static stockpiles, although this depends on the antibiotic residue and their interactions. Similarly, thermophilic anaerobic digesters operating under steady-state conditions may be more effective at mitigating antibiotic residues than mesophilic or irregularly operated digesters or anaerobic lagoons. The number of ARB may decline during composting and digestion or be enriched

as the bacterial communities in these systems shift, affecting relative ARG abundance or acquire ARG during treatment. Antibiotic resistance genes often persist through these systems, although optimal management and higher operating temperature may facilitate their mitigation. Less is known about other manure treatments, although separation technologies may be unique in their ability to partition antibiotic residues based on sorption and solubility properties. Needed areas of study include determining natural levels of AR in dairy systems, standardizing and optimizing analytical techniques, and more studies of operating on-farm systems, so that treatment system performance and actual human health risks associated with levels of antibiotic residues, ARB, and ARG found in dairy manure can be accurately assessed.

Key words: anaerobic digestion, antimicrobial, compost, milk cow, solid-liquid separation

US DAIRY AND ITS POTENTIAL IMPACT ON ANTIBIOTIC RESISTANCE

Antibiotics are as critical to the maintenance of food-producing animals, including dairy cattle, as they are to human health, but the effectiveness of these important therapies is threatened by the proliferation of antibiotic resistance (AR). Although antibiotic-resistant bacteria (ARB) and their antibiotic resistance genes (ARG) have existed for millennia, their prevalence has rapidly increased in conjunction with widespread manufacturing and anthropogenic use of antibiotics (D'Costa et al., 2011). Although selective pressures and expression triggers were historically minimal (Aminov, 2009), AR has proliferated with the unintentional, but consequential, release of antibiotic residues, ARB, and ARG into the environment from various anthropogenic sources (Pruden et al., 2013), including dairy (Figure 1).

The exact impact of antibiotic use by dairy operations to the proliferation of AR is unclear (Oliver et al.,

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2011), though links between AR in cattle and human infections have been documented (HHS-FDA-CVM, 2012; Casey et al., 2013; Madec et al., 2017). One potential AR transmission route is the dairy production chain (Papadopoulos et al., 2018), but in the United States a federally mandated, rigorous human food safety system ensures that milk and meat are antibiotic residue- and pathogen-free. These regulations require all veterinary antibiotics to be federally approved, properly labeled, and administered accordingly with veterinarian oversight (HHS-FDA-CVM, 2012, 2013; FDA, 2018). The milk and meat from a treated animal must be “withheld” from the human food supply for a treatment-dependent length of time (days to weeks) to ensure they are safe for human consumption (FDA, 2016). Furthermore, established legal frameworks require farm-, processing- and retail-level testing of these foods (FDA, 2015). In 2017, no inspected retail

samples, only 0.02% of all 3.82 million milk truck loads (GLH Inc., 2018), and only 0.4% of the 100,275 cattle carcasses tested were in violation (USDA/FSIS/OPHS, 2017) and removed from the human food supply.

Consequently, a perhaps more likely AR transmission route is the spread of antibiotic residues, ARB, and ARG from dairy manure (urine + feces) to croplands and natural systems, which may then serve as AR reservoirs and vectors to human pathogens (Lupo et al., 2012; Economou and Gousia, 2015; Xie et al., 2018). Studies show that dairy pastures (López et al., 2012), calf hutch areas (Li et al., 2014), land near farmsteads, and field soils exposed to dairy manure may all have elevated levels of antibiotic residues, ARB, and ARG (Heuer et al., 2011; Peng et al., 2017; Pollard and Morra, 2018). Elevated levels may also be found in irrigation water (Blaustein et al., 2016; Hafner et al., 2016; Palacios et al., 2017), surface waters (Pruden et al., 2012;

Figure 1. Anthropogenic sources of antibiotic resistance and potential transmission routes to human populations. The figure was adapted, with permission, from a graphic published by the Food Animal Concern Trust (FACT, 2018; <https://www.keepantibioticsworking.org/>), and expanded to include a more complete scope of possible transmission routes.

Li et al., 2014; Kulesza et al., 2016), and groundwater in the vicinity of dairy operations (Li et al., 2015; Pan and Chu, 2017b; Spielmeyer et al., 2017). Soil bacteria exposed to dairy manure can become reservoirs of ARG (Yang et al., 2006; Edrington et al., 2009; Fahrenfeld et al., 2014), as can their bacteriophages (Ross and Topp, 2015). Moreover, crops fertilized with cattle manure can accumulate antibiotic residues (Tasho and Cho, 2016; Pan and Chu, 2017a,c) and ARG (Marti et al., 2013, 2014; Tien et al., 2017).

Along with other anthropogenic sources of AR, such as municipal wastewater treatment plants whose effluents and biosolids have similar environmental consequences (Pruden et al., 2012), the dairy industry has an opportunity and responsibility to reduce their potential contribution to AR. Not only is this essential to the support of the One Health initiative (Robinson et al., 2016), but it should be economically valuable as well. Dairy operations in the United States produce around nearly 100 billion kilograms of milk per year (NASS, 2018b), worth \$34 billion (NASS, 2017b), and culled milk cows constitute over 10% of the US beef supply (NASS, 2018a). The presence of antibiotic residues may undercut economics and efforts by the US dairy industry to help meet global demands for high-quality food (Groot and van't Hooft, 2016) produced in a socially and environmentally responsible manner (US Dairy, 2018).

Manure management systems offer a potentially important on-farm control point for AR (Pruden et al., 2013). A catalog of more than 250 scored manure management technologies is available to US dairy operations (Newtrient, 2018) for review, although AR mitigation was not part of the scoring of each technology. While this is a growing area of research, how even the most common dairy manure management systems handle antibiotic residues, ARB, and ARG is still not well understood (Franklin et al., 2016). Here, we provide a comprehensive, non-alarmist synthesis of the literature dealing with AR in dairy manure and dairy manure treatment systems. Specifically, we summarize (1) antibiotic use by US dairy cows; (2) the presence of antibiotic residues, ARB, and ARG in untreated dairy manure; (3) the effects of common manure management systems on AR; and (4) the AR mitigation potential of field treatment systems.

USE OF ANTIBIOTICS ON US DAIRY OPERATIONS

It is difficult to directly compare the use of medically important antibiotics between humans and animals because of limited access to and discrepancies in the methods by which hospital, medical clinic, and farm records are kept. Sales data suggest that roughly three-fourths of total US antibiotic usage (>8 million kilograms per

year) are consumed by livestock and poultry, although only 60% of the antibiotics used by farms are medically important (FDA, 2017). Global estimates suggest that swine and poultry operations are the primary consumers of antibiotics (Van Boeckel et al., 2015), but in the United States, most (43%) of the medically important antibiotics used by animal agriculture were administered to beef and dairy cattle (FDA, 2017).

Based on the most recent census data, over 60,000 dairy operations managed the US herd of ~17.5 million dairy cattle (NASS, 2014), and most used antibiotics. According to national surveys, 90% of US dairy operations used antibiotics as therapeutics for cows, 25% as prophylactics for heifers, and 4% for pregnant heifers (NAHMS, 2018). Note that dry-cow antibiotic treatments, which are used by around 90% of US dairy operations on all cows during dry off (NAHMS, 2008), were considered therapeutic, not prophylactic, as they are used to eliminate intramammary infections and to prevent new infections. Only 4.3% of operations (2.8% of cows) are certified organic (NASS, 2017a,c), a production system that prohibits antibiotic use and requires treated animals to be segregated and sold (NOP, 2018). Amish and Mennonite dairy operations, which represent another small subset of the industry, use antibiotics similarly to conventional operations (Schewe and Brock, 2018). Of the antibiotics approved for dairy operations, β -lactams (penicillins and cephalosporins) and tetracyclines are the most common therapeutics, followed by macrolides, sulfonamides, and florfenicol, with tetracyclines and, until the recent change in their labeling, sulfonamides, being the most common prophylactics (NAHMS, 2018; Table 1).

The need to safeguard antibiotics and reduce production costs are driving industry-wide efforts, such as the National Dairy Farmers Assuring Responsible Management (FARM) initiative (FARM, 2018), to improve antibiotic stewardship. One farm-level initiative is improved pathogen diagnostics to optimize antibiotic therapy selection and avoid nonbeneficial use (Lago et al., 2011). Selective mastitis treatments, for example, may reduce the number of antibiotic treatments for mastitis by 68%, while maintaining the same herd health standards (Vasquez et al., 2017). Carefully selecting antibiotic therapies that are used together may also help fight the spread of AR, as antagonistic interactions between certain antibiotics can be used to counteract the development of drug resistance (Baym et al., 2016). Other farm-level initiatives include disease prevention through improved animal husbandry (Kleinlützum et al., 2013), housing, and ventilation (Lorenz et al., 2011a; Phillips et al., 2013), and careful management of transition (pre- to postcalving) cows (Lorenz et al., 2011b; Love et al., 2016). Recent federal- (HHS-FDA-

Table 1. Antibiotics approved for dairy cattle and their critical importance according to the World Health Organization (WHO, 2017)¹

Drug class	Antibiotic	WHO categorization	Trade name(s)	Labeled for ²				
				Calves	Heifers	Lactating cows	Dry cows	Other ³
Aminocoumarin Amino-glycosidases	Novobiocin ⁴	None	AlbaDry Plus				×	
	Dihydrostreptomycin sulfate	Critically imp.	Quartermaster				×	
Amphenicol Cephalosporins	Neomycin	Critically imp.	Neomycin	×	×			
	Spectinomycin sulfate	Important	Adspec	×	×			
	Spectinomycin sulfate	Important	Linco-Spectin					×
	Florfenicol ⁴	Highly imp.	Nuflor, Resflor	×	×	×		
		Critically imp.	Excede	×	×	×		
	Ceftiofur hydrochloride ⁴	Critically imp.	Excenel					
	Ceftiofur hydrochloride ⁴	Critically imp.	Spectramast DC			×	×	
	Ceftiofur hydrochloride ⁴	Critically imp.	Spectramast LC			×	×	
	Ceftiofur sodium ⁴	Critically imp.	Ceftiflex, Naxcel	×	×	×		
	Cephapirin sodium	Highly imp.	ToDay			×		
Lincosamides	Lincomycin hydrochloride	Highly imp.	Linco-Spectin					×
Macrolides	Pirlimycin hydrochloride	Highly imp.	Pirsue					
	Erythromycin	Critically imp.	Erythromycin	×	×	×		
	Tildipirosin ⁴	Critically imp.	Zuprevo					
	Tilmicosin	Highly imp.	Micotil 300, Pulmotil 90	×	×			
	Tulathromycin ⁴	Critically imp.	Draxxin	×	×			
	Tylosin ⁴	Critically imp.	Tylan 50, Tylan 200, TyloVed	×	×			
	Amoxicillin trihydrate	Critically imp.	AmoxiMast					
	Ampicillin	Critically imp.	Polyflex	×	×	×	×	
	Cloxacillin benzathine	Highly imp.	Dryclox, Orbenin					
	Hetacillin potassium	Critically imp.	Hetacin, Polymast			×	×	
Penicillins	Penicillin G	Critically imp.	Agri-Gillin, Bactracillin G, Norocillin, PenAqueous, Penicillin, Penject, PenOne, ProPen	×	×	×		
			AlbaDry Plus, Quartermaster					
	Penicillin G	Critically imp.	AlbaDry Plus, Quartermaster					
	Sulfadimethoxine	Highly imp.	Albon, Dimethox, Sulfadimethoxine, Sulfamed, Sulfasol	×	×	×		
	Sulfamethazine	Highly imp.	Aureo S 700, SMZ 454, SupraSulf, Sustain III	×				
	Chlortetracycline	Highly imp.	Aureo S 700, Aureomycin, Chlormax, Chloronex, CLTC 100, Pennchlor 64	×	×			
	Oxytetracycline	Highly imp.	Agrimycin, Duramycin, Liquimycin, Noromycin, Oxybiotic, Oxytet 100, Pennox, Terra-Vet, Terramycin, Tetroxy 343, Vetrimycin	×	×			
	Oxytetracycline	Highly imp.	Bionycin 200, Liquamycin LA 200, Tetracycline SP 324 Powder	×	×	×	×	
	Enrofloxacin ⁴	Critically imp.	Baytril, Enroflox 100	×	×			

¹Categorizations are based on 2 criteria: (1) the antibiotic is the sole, or limited, therapy to treat serious bacterial infections in humans, and (2) the antibiotic is used to treat infections in humans caused by bacteria from nonhuman sources or by bacteria that may acquire resistance from nonhuman sources. Critically important (imp.) antibiotics meet both criteria, highly important antibiotics meet 1 of the 2 criteria, and important antibiotics are used in humans but meet neither criterion. Designations are made at the class level.

²Can be used in nonlabeled animal classes providing *Animal Medicinal Drug Use Clarification Act* of 1994 (AMDUCA) is followed.

³Used as a cattle semen preservative.

⁴Veterinary usage only.

CVM, 2012, 2013) and state-level mandates (California Senate, 2015; Maryland Senate, 2017) also require more prudent on-farm antibiotic usage. The drug approval process has also been tightened and now requires AR and environmental impact assessments, such as residue excretion rate and environmental mobility testing (FDA, 2018).

ANTIBIOTIC RESISTANCE IN UNTREATED DAIRY MANURE

Antibiotic Residues and Metabolites in Dairy Manure

Studies of excretion rates in dairy animals are currently limited but ultimately depend on the antibiotic, dosage, age and condition of the animal, and time since treatment, with the concentration in the manure also affecting detection (Spielmeyer et al., 2014). Antibiotics are effective at extremely low levels but, due to inertness, in vivo large dosages are administered to enable adequate drug delivery (Levison and Levison, 2009), which may result in up to 90% of the dosage being excreted (Sarmah et al., 2006). Ince et al. (2013) reported that 20% of an 8,800-mg i.m. injection of oxytetracycline was detected in pre-excreted feces (cecum samples) from a Holstein cow over a 20-d period, most in the days immediately after treatment. This estimate did not account for bioactive antibiotic metabolites or transformation products commonly produced in vivo (Higuchi, 1963; Sokoloski et al., 1977) or the fraction excreted in urine. In a study of 3 end-of-lactation Holstein dairy cows administered 300 mg of cephalixin per quarter, less than 1% of the dose was found in feces, but more than 50% was excreted in the urine as desacetyl cephalixin (Ray et al., 2014), a metabolite that maintains up to 55% of the antibiotic activity of cephalixin (Jones and Packer, 1984). The lincosamide antibiotic pirlimycin will also transform in vivo, with a conjugate excreted at levels up to 50% of the initial dosage in dairy (Hornish et al., 1992, 1998) and beef cattle (Arikan et al., 2006).

Often, studies do not consider excretion rates in feces and urine but instead focus on antibiotic concentrations in manure. Tetracycline residues are the best studied and perhaps most prevalent, though methodological bias may exist as these antibiotics are relatively easy to detect (Wallace and Aga, 2016). Tetracycline, chlortetracycline, and oxytetracycline are most commonly measured in dairy manure at concentrations of approximately 5, 10, and 250 $\mu\text{g kg}^{-1}$ dry manure, respectively (Storteboom et al., 2007; Watanabe et al., 2010). Interestingly, these concentrations are substantially lower than those reported in studies of European and Asian dairy manures (Table 2), which may attest to country-

level differences in antibiotic practices. With optimized extraction and analytical approaches, Wallace and Aga (2016) were able to detect additional tetracycline residues in US dairy manures and show that they were primarily associated with manure solids and only limitedly solubilized in manure liquids.

Unlike tetracyclines, the widely used β -lactam antibiotics are more soluble and seemingly less persistent (Table 2). In our study of over 80 fresh manure samples collected from 11 northeastern US dairy operations over a 15-mo period, no β -lactam residues were detected in any manure samples, despite the common use of penicillins and cephalosporins on these dairy operations (Oliver et al., 2017). To our knowledge, the only study of dairy cows to measure a β -lactam residue found very low levels of cephalixin and only in pre-excreted cecal samples and cow urine collected with a catheter (Ray et al., 2014). The apparent instability of β -lactams in excreted manure and detection challenges associated with analyzing their degradation products may explain the discrepancies between usage and detection (Deshpande et al., 2004).

Sulfonamide, macrolide, and lincosamide residues have also been measured in US dairy manure samples but are infrequently detected and typically at very low (<1 ppb) concentrations. On occasion, however, higher concentrations, up to 430 $\mu\text{g kg}^{-1}$ dry manure of certain sulfonamides and up to 30 $\mu\text{g kg}^{-1}$ dry manure of certain macrolides, have been measured (Watanabe et al., 2010; Wallace and Aga, 2016), similar to findings from other countries (Table 2).

ARB in Conventional and Organic Dairy Manure

The US Centers for Disease Control has identified the most clinically dangerous ARB (CDC, 2013). Those likely associated with dairy operations included antibiotic-resistant *Enterobacteriaceae* (specifically nontyphoidal *Salmonella*), antibiotic-resistant *Campylobacter*, methicillin- and vancomycin-resistant *Staphylococcus*, and vancomycin-resistant *Enterococcus*. Most dairy manure studies of ARB, however, have focused on food-borne pathogenic strains of bacteria; namely, *Escherichia coli* and *Salmonella* spp. (Feßler and Schwarz, 2017). According to the US National Antibiotic Resistance Monitoring System (NARMS), 15% or fewer of the pathogens isolated from dairy cow cecal samples were resistant to an antibiotic (NARMS, 2018), though published works have reported higher prevalences (Table 3).

Tetracycline resistance is most common in *E. coli* isolated from US dairy manure (Table 3). Although tetracyclines are widely used by US dairy operations, the effect of antibiotic usage on the shedding of

Table 2. Antibiotic concentrations ($\mu\text{g kg}^{-1}$ of dry manure) detected in dairy manures from various regions/countries (blank cells indicate a lack of data)

Antibiotic class	Antibiotic compound	United States	Europe	China	Japan
Tetracyclines	Anhydrochlorotetracycline	0–141 ^{1,2}			
	Anhydrotetracycline	0–200 ^{1,2}			
	Chlortetracycline	0–107 ^{1–4}	20–500 ^{7,8}	240–27,590 ^{14,15}	0–1.3 ¹⁶
	Doxycycline		0–20 ^{7,8}	440–1,050 ¹⁴	
	Epichlorotetracycline	0–71 ^{1–3}			
	Epitetracycline	0–1,100 ^{1–3}			
	Oxytetracycline	0–556 ^{1–4}	0–871,700 ^{7–13}	210–103,700 ^{14,15}	0–1.0 ¹⁶
	Tetracycline	0–1,200 ^{1–4}		430–26,900 ¹⁵	0–1.2 ¹⁶
Beta-lactams	Cephapirin	0–480 ^{1,5}			
	Ceftiofur	0 ¹			
	Amoxicillin	0 ¹			
	Ampicillin	0 ¹			
	Penicillin G	0 ¹			
	Penicillin V	0 ¹			
Fluoroquinolone	Ciprofloxacin			280–29,590 ^{14,15}	2.2–12 ¹⁶
	Danofloxacin			410–3,060 ¹⁴	
	Difloxacin			160–2,630 ^{14,15}	
	Enrofloxacin			460–46,700 ^{14,15}	
	Fleroxacin			0–2,200 ¹⁴	
	Levofloxacin				1.1–2.1 ¹⁶
	Lomefloxacin			610–5,530 ¹⁴	
	Norfloxacin			430–2,760 ^{14,15}	
Sulfonamides	Sulfachloropyridazine			150–360 ^{14,15}	
	Sulfadiazine			0 ¹⁴	
	Sulfadimethoxine	0–60 ^{1–3}			
	Sulfadimidine			100–180 ¹⁴	
	Sulfaguanidine			50–300 ^{14,15}	
	Sulfamerazine	0 ¹		90–110 ^{14,15}	
	Sulfamethazine	0–430 ^{1–3}			
	Sulfamethoxazole	0–36 ³		0–1,020 ^{14,15}	10–37 ¹⁶
	Sulfamonomethoxine			60–300 ^{14,15}	0–22 ¹⁶
	Sulfanilamide			0–80 ¹⁵	
Macrolides	Azithromycin				
	Clarithromycin	0–0.01 ¹			
	Erythromycin	<1,000 ^{1–3}			
	Roxithromycin	0 ¹			
	Tilmicosin	0–29 ^{1–3}			
	Tylosin	0 ^{1–4}	100–115,500 ⁸	220–280 ¹⁵	
Ionophores (not medically important)	Amprolium	<100 ⁶			
	Lasalocid	0–3,300 ⁶			
	Monensin	0–1,000 ^{4,6}			
	Salinomycin	<100 ⁶			

¹Unpublished data from a 15-mo study of 11 dairy farms in the northeastern United States conducted by the authors.²Wallace and Aga (2016).³Watanabe et al. (2010).⁴Storteboom et al. (2007).⁵Ray et al. (2014).⁶Arikan et al. (2016).⁷Carballo et al. (2013).⁸Kemper (2008).⁹De Liguoro et al. (2003).¹⁰Akyol et al. (2014).¹¹Akyol et al. (2016a).¹²Ince et al. (2013).¹³Türker et al. (2013).¹⁴Zhao et al. (2010).¹⁵Li et al. (2013).¹⁶Motoyama et al. (2011).

Table 3. Prevalence (% of tested isolates) of antibiotic-resistant bacteria in US dairy manure samples by species and antibiotic (blank cells indicate a lack of data)

Antibiotic class	Antibiotic compound	<i>Escherichia coli</i>	<i>Salmonella</i> spp.	<i>Campylobacter</i> spp.	<i>Enterococcus</i> spp.	<i>Staphylococcus</i> spp.
Aminocoumarin Aminoglycoside	Penicillin/novobiocin					1.3 ⁹
	Amikacin	0 ¹	0.0 ^{5,6}			
Cephalosporin	Apramycin	0.9 ¹				
	Clindamycin			1.0–9.1 ^{2,8}		
	Gentamicin	0–2.6 ^{1,2}	0–4.4 ^{2,5,6}	0–1.8 ^{2,8}	0–1.4 ²	
	Kanamycin	0–14 ^{1,2}	0.7–31 ^{5,7}	30–32 ⁸	0–2.9 ²	
	Neomycin		26 ⁶			
	Spectinomycin		14 ⁶			
	Streptomycin	6.3–15 ^{1,2}	9.0–44 ^{2,5,6}	0.6–1.6 ⁸	0–5.6 ²	
	Cefoxitin	0.9–1.5 ^{1,2}	3.7–40 ^{2,5,6}			
	Ceftiofur	0.6–6.5 ^{1,3}	4.4–34 ^{2,5,6}			
	Ceftriaxone	0–2.8 ^{1,2}	0–39 ^{2,5,6}	1.4–2.3 ⁸		
Chloramphenicol Fluoroquinolone	Cephalothin	4.1 ¹	4.8–8.8 ^{5,7}			
	Chloramphenicol	2.3–4.0 ^{1,2}	4.4–37 ^{2,5,6}	0–1.1 ⁸	0–1.3 ²	
	Ciprofloxacin	0–0.9 ^{1,2}	0–0.3 ^{2,5,6}	0.9–47 ^{2,8}	0–50 ²	
	Enrofloxacin		0.6 ⁶			
	Florfenicol	37 ⁶	3 ⁶	0–0.3 ^{2,8}		
Glycylcycline Glycopeptide Ketolide Lincosamide Lipopeptide Macrolide	Nalidixic acid	0–1.8 ^{1,2}	0–0.4 ^{2,5,6}	1.3–47 ²	0 ²	
	Tigecycline				0 ²	
	Vancomycin			0.3–7.4 ²	0–99 ²	
	Telithromycin				1.4–1.9 ²	
	Lincomycin					
	Daptomycin					
	Azithromycin	0–0.6 ²	0–0.5 ²	0–9.1 ²	0–8.7 ²	0.8 ⁹
	Erythromycin			0.3–9.1 ^{2,8}	1.3–10 ²	
	Tylosin				0 ²	
	Nitrofurantoin				0 ²	
Nitrofurantoin Oxazolidinone Penicillin	Linezolid					
	Amoxicillin/clavulanic acid	1.3–1.8 ^{1,2}	4.8–40 ^{2,5,6}	0–0.1 ²		0.4 ⁹
	Ampicillin	1.8–13 ^{1–4}	4.4–42 ^{2,5,6}			1.3 ⁹
	Penicillin			7.1–8.6 ²	0 ²	1.3 ⁹
	Penicillin/novobiocin					
Streptogramins Sulfonamide	Quinupristin/dalfopristin	14 ¹	3.7–11 ^{5,7}	37–39 ²	1.3–8.8 ²	
	Sulfamethoxazole	5.5–11 ²	9.0–11 ²			
	Sulfamethoxazole/sulfisoxazole		72 ⁶			
	Sulfadimethoxine		44 ⁶			
	Sulfisoxazole		0–6 ^{2,5,6}			19 ⁹
	Trimethoprim/sulfamethoxazole	0–5.0 ^{1,2}	41 ⁶			
	Chlortetracycline	6.7–26 ^{1–4}	12–44 ^{2,5,6}	49–76 ^{2,8}	7.1–45 ²	2.9 ⁹
Tetracycline	Tetracycline		42 ⁶			
	Oxytetracycline	0.5–8.7 ⁴	0.5–8.7 ⁴	8.6 ⁷		
Resistant to >5 antibiotics						

¹Sato et al. (2005).²NARMS (2018).³Mann et al. (2011).⁴Savant et al. (2007).⁵Blau et al. (2005).⁶Cummings et al. (2013).⁷Ray et al. (2007).⁸Halbert et al. (2006).⁹Abdi et al. (2018).

antibiotic-resistant *E. coli* remains unclear. In a comparison of conventional and organic dairy operations, the prevalence of antibiotic-resistant *E. coli* was only higher on conventional farms for some of the antibiotics tested, whereas resistance was more prevalent on organic operations for one of the antibiotics, and production system (organic or conventional) had no effect on resistance to the most widely used cephalosporin antibiotics (Sato et al., 2005). Other extensive studies of fecal *E. coli* isolates also noted farm- and herd-level discrepancies between usage of ceftiofur and *E. coli* resistance to cephalosporins (Tragesser et al., 2006; Mann et al., 2011). The inability to correlate ARB and antibiotic usage could relate to the timing of sampling (Singer et al., 2008; Chambers et al., 2015), sub-strain-level differences (McConnel et al., 2016), and horizontal gene transfer complexities (Dolejska et al., 2011; Gonggrijp et al., 2016), including the arrival of ARB onto farms from nonfarm sources, exemplified by the spread of CTX-M *E. coli* strains onto Washington dairy operations from outside human populations (Davis et al., 2015; Afema et al., 2018). Interestingly, in other countries, antibiotic-resistant *E. coli* are often associated with dairy operation antibiotic use (Carballo et al., 2013; Santman-Berends et al., 2017; Obaidat et al., 2018a), but the higher prevalence of antibiotic-resistant *E. coli* found in these studies may be driving this correlation (Parin et al., 2018).

The prevalence of antibiotic-resistant *Salmonella* in US dairy manure is similar to that of *E. coli* (Table 3). In a large, multistate study of dairy herds, 12% of 3,709 fecal *Salmonella* isolates were resistant to at least one antibiotic and 5% were multidrug resistant (Blau et al., 2005). The prevalence of antibiotic-resistant *Salmonella* may be declining, however. According to repeated sampling at 6 Michigan dairy operations, resistance to at least one antibiotic decreased from 15% in 2000 to <1% in 2009 (Habing et al., 2012). This decrease was concurrent with an increase in *Salmonella* shedding, suggesting possible displacement of resistant populations by susceptible ones. A large study of veterinary clinical samples collected from northeastern US dairy operations from 2004 to 2011 similarly noted a significant decrease in the prevalence of resistant *Salmonella* isolates (Cummings et al., 2013). Another indication of this decline comes from a recent study of all animal groups (cows, heifers, and calves) on 80 dairy operations in Pennsylvania, where 99% of the 1,095 isolates from ~450 manure samples were pan-susceptible to a panel of 14 antibiotics (Cao et al., 2017). Generally, across studies, antibiotic-resistant *Salmonella* were more common on larger farms and associated with warmer sampling months and locations at lower latitudes. Interestingly,

it has been shown that the susceptibility of *Salmonella* isolates is similar between organic and conventional operations for most antibiotics tested when controlling for herd size and location (Ray et al., 2006).

Fewer dairy manure studies have focused on other genera, such *Campylobacter* and *Enterococcus*, which are generally susceptible to antibiotics (NARMS, 2018), though tetracycline and lincomycin resistance can be prevalent depending on the species (Table 3). It is noteworthy that some studies have found significant resistance of *Campylobacter* isolates to kanamycin (Halbert et al., 2006). Kanamycin is not a therapy for cows, but ARG encoding kanamycin and tetracycline resistance (*kanR* and *tetO*) can be co-localized on mobile genetic elements in *Campylobacter* spp. (Tenover et al., 1992) and other pathogens such as *E. coli* (Badrinarayanan et al., 2012). This raises questions about potential links between tetracycline use on farms and selection of resistance to kanamycin, which is a treatment of last resort for tuberculosis patients (Shim and Jo, 2013).

Most investigations of *Staphylococcus* spp. have focused on mastitis-infected milk, not manure. However, waste milk, if not pasteurized and fed to calves, is often discarded into manure and would thus have a similar fate to manure-based pathogens. In a study of 239 *Staphylococcus* isolates from 187 mastitis-affected cows at 33 dairy operations, 34% were resistant to at least one of the 10 antibiotics screened (Abdi et al., 2018). Those authors noted that the prevalence of ARB varied with farm, time, and type of antibiotic, with an unexpectedly high level of resistance to sulfadimethoxine and increasing resistance to tetracycline, neither of which are typically used as a mastitis treatment. Work on herds overseas has found antibiotic resistance in *Staphylococcus* spp. to be only occasionally elevated (Obaidat et al., 2018b; Ronco et al., 2018) but still herd specific (Beyene et al., 2017; Nobrega et al., 2018). There is also some evidence that conversion to antibiotic-free production may reduce the prevalence of antibiotic-resistant *Staphylococcus*, particularly β -lactam resistance (Park et al., 2012).

The NARMS databases are beginning to develop the nationwide level of understanding needed to accurately comprehend the prevalence of ARB on US dairy operations, but analysis of the meta-data required to determine patterns of ARB dissemination is still in its infancy. There continue to be limitations associated with the lack of standardized surveillance protocols and sampling bias; that is, studies based on samples submitted to clinics, which may not capture healthy asymptomatic carriers of ARB. More effort is needed to understand the natural ARB populations existing in both conventional and organic herds so that the effect

of treatments on ARB can be better understood. Until these baseline-level monitoring efforts are established, understanding the changing prevalence of ARB and their potential effect on the spread of AR will continue to be limited.

ARG in Untreated Dairy Manure

Cattle manures are also reservoirs of more than 60 different ARG (Qian et al., 2018), with resistomes varying from herd to herd (Wichmann et al., 2014). In dairy cow manure, ARG can be associated with bacteria and their phages and prophages, and can also exist extracellularly on plasmids and transposable elements (ESA, 2014). Most studies conducted have not separated these different ARG populations because of the technical challenges in doing so, although ARG associated with plasmids, transposable elements, and phages may be more likely to spread AR (Vikesland et al., 2017).

Some recent studies have focused on *E. coli*-associated ARG and confirmed herd-level resistome variability, sometimes with differences in ARG encoding resistance to the same antibiotic (Cao et al., 2017). Tetracycline resistance may be the most common, with a sequencing effort of 160 antibiotic-resistant *E. coli* and 28 fecal metagenomes indicating that 61% of all identified ARG encoded resistance to tetracycline (Haley et al., 2017). Similar findings were found in our recent study of 11 northeastern US dairy operations (Hurst et al., 2019). The dominance of tetracycline resistance genes was actually noted as an impediment to measuring the effect of ceftiofur treatments on total ARG abundance in one study of cow fecal samples (Chambers et al., 2015), although sample timing and baseline determinations can complicate metagenomics analysis of manure-associated ARG (Caudle, 2014).

Understanding baseline ARG levels is challenged by the diversity of dairy animal resistomes (Santamaria et al., 2011; Kyselková et al., 2015a, 2016), even in young cattle that have not received antibiotic treatments (Durso et al., 2011). Complicating this is the fact that calves can rapidly develop ARB populations following initial antibiotic treatments (Pereira et al., 2014a), and ARG can be transmitted to young calves that are fed milk from treated cows (Pereira et al., 2014c, 2016), from animals raised at off-farm facilities (Pereira et al., 2015), or spread through the farm via animal contact (Pereira et al., 2014b), feed and water (Yang et al., 2006), flies (Xu et al., 2018), or dust and aerosols (McEachran et al., 2015; Sancheza et al., 2016). Despite the challenges, whole-genome sequencing will help facilitate the broad surveillance needed to monitor unbiased sample populations so the impact of

environment and herd management practices on ARG can be resolved (Baker et al., 2018). To focus these efforts, the USDA Agricultural Research Service's Agricultural Antibiotic Resistance Network (AgAR; USDA, 2018) has proposed 4 standard gene targets (*intI1*, *ermB*, *ctx-m*, and *sul1*) based on human health and farm relevance criteria (USDA, 2018). Standardized surveillance of cattle, dairy agroecosystems, and cattle-free environments is needed to differentiate pre-existing ARG prevalence from increased prevalence caused by farming and antibiotic use practices.

Even as herd health surveillance efforts are initiated and other efforts to optimize antibiotic administration are made, the need remains to manage the residues, ARB, and ARG that are inevitable present in the manure of treated animals. As stated earlier, manure management systems offer a potentially important on-farm control point for AR, even though no current systems or treatment components are specifically designed to mitigate AR; therefore, their efficacy is currently opaque. In the following sections, we review some of the most common manure handling and treatment systems and their impact on AR. Efforts are made to relate scientific findings to practical management opportunities and to identify needed areas of research so that mitigation goals can be achieved.

SOLID-LIQUID SEPARATION

Solid-liquid separation (SLS) is used by many US dairy operations to reclaim fibrous "separated solids" from dairy manure slurries for various purposes (e.g., to prevent accumulation in long-term storages or for reuse as animal bedding). Differences in the chemical properties of antibiotic residues affect their solubility and sorption properties, and thus their partitioning between separated solid and liquid fractions of manure. For example, Wallace and Aga (2016) showed that tetracyclines and macrolides preferentially partitioned with centrifuge-separated solids. Hurst et al. (2018) similarly found that most of the non-medically important ionophores were sorbed to manure solids, except for monensin, which was highly soluble and detectable in filtrate following membrane purification of dairy manure. Centrifugation and membrane purification are not common SLS strategies used by US dairy operations however; bar screens and screw-presses are more widely used. These SLS systems only partially fractionate manure, with separated solids remaining wet (60–80% moisture content) and separated liquids retaining up to 10% of total solids on a wet basis (Oliver et al., 2018). Solids capture efficiencies are also variable (5–60%). Thus, different SLS technologies, depending on influent

characteristics, will partition more or less of the total solids and their associated antibiotic residues. Recent investigations have suggested that some SLS systems may mitigate bovine pathogens (Liu et al., 2017) and ARG (Tien et al., 2017; Wallace et al., 2018), but these studies lack the mass-flow and influent characterization data required to completely assess system performance. Future assessments of the AR mitigation potential of SLS systems must consider these important metrics to properly validate mitigation. Such studies are needed, because SLS systems may offer an effective means of concentrating manure-associated antibiotic residues, ARB, and ARG for advanced processing using a form of treatment capable of possible mitigation.

STOCKPILING AND COMPOSTING

The simplest form of manure storage is stockpiling—the heaping of solid manure, soiled bedding, or separated solids. Stockpiling is typically used for short-term (weeks to months) storage of small quantities of dairy manure to apply to cropland at times based on crop nutrient uptake and field accessibility, although some dry lot dairies in arid climates use stockpiling for year-round manure storage. As stockpiles age, they degrade. By incorporating dry matter, managing moisture content, and mixing/aerating, more aerobic and higher temperature conditions can facilitate a composting process (Hubbe et al., 2010). Although stockpiling can reduce some antibiotic residues, ARB, and ARG, more intensively managed composts are generally more effective (Youngquist et al., 2016), though this depends on composting conditions. Again, mitigating AR is not the aim of current compost or stockpile management systems.

Degradation of Antibiotic Residues During Stockpiling and Composting

Degradation of antibiotic residues in cow manure stockpiles and composts can take weeks, months, or longer, depending in part on the antibiotics. In stockpiled Holstein cattle manure, Sura et al. (2014) found that chlortetracycline declined rapidly, with <1% of the starting concentration detectable after 17 d. The time required for the concentration to decline to half of the initial concentration (DT_{50}) was 1.8 d. For sulfamethazine, degradation was slower (DT_{50} = 20.8 d) and incomplete (2% remained after 77 d). Like chlortetracycline, there was an initial and rapid decrease in tylosin (DT_{50} = 4.7 d), but then degradation slowed, with 13% of the residue still remaining after 140 d.

Similar compound-dependent degradation rates have been reported in dairy manure composts for other medically important antibiotics (Ray et al., 2017) and ionophores (Arikan et al., 2016). Some US dairies may also utilize compost bedded packs for their sick cows or entire herds (Barberg et al., 2007). To our knowledge, this form of bedding management as it relates to AR has not been studied, although, in principle, what is reviewed below about AR in dairy manure composts should apply to well-managed compost bedded packs.

Interactions between different antibiotic residues also affect degradation rates in stockpiles and composts. When chlortetracycline and sulfamethazine were both present in a stockpile, Sura et al. (2014) found that the degradation rate of chlortetracycline slowed, taking 56 d (DT_{50} = 6.0 d) instead of 17 d to decline (DT_{50} = 1.8 d). This was attributed to potential inhibition of chlortetracycline-degrading bacteria by sulfamethazine. Conversely, both Storteboom et al. (2007) and Arikan et al. (2009) found enhanced degradation rates when multiple antibiotics were present in dairy, beef, or horse manure composts. As these synergistic effects were observed for antibiotics of the same class (tetracyclines), it was hypothesized that compost microbes may have acclimated to act on multiple compounds. Understanding these antibiotic interactions has practical value. Dairies in the United States often segregate treated animals but handle their manure collectively. There may be an opportunity to only combine treated animal manure when complementary antibiotic residues are expected and to keep this manure separate when anticipated residues are likely to impair degradation.

Influence of Composting Conditions on Antibiotic Residue Degradation

It is also important that stockpiles and composts are properly managed to optimize degradation rates and minimize leachate of antibiotic residues (Dolliver et al., 2008). Typically, faster degradation has been associated with more intensively managed composts. By adding bulking material, managing moisture content, and mixing/aerating, Storteboom et al. (2007) were able to increase degradation rates of several tetracyclines in dairy and beef manure stockpiles, sometimes reducing DT_{50} values by half. Similar patterns were noted for tetracyclines in well-managed cattle manure stockpiles (Arikan et al., 2009) and for the macrolide kitasamycin in a cow manure and sawdust compost (Ding et al., 2014). In apparent contrast, the work by Sura et al. (2014), which compared stockpiles to mixed windrows, found that degradation slowed with mixing. This may have been due to disruption of the antibiotic-degrading

microbial community in the windrows from the drying and temporary cooling associated with the frequent mixing, but could also be an artifact associated with the high initial starting concentrations of antibiotics. Antibiotic degradation can also be elevated under thermophilic (~55°C) versus mesophilic (~37°C) compost conditions (Mitchell et al., 2015b), likely due to differences in the selected microbial communities.

Nonbiological Degradation of Antibiotics in Compost

Most studies have not segregated the nonbiological degradation of antibiotics in composts from the biological. First, some antibiotic molecular structures are relatively unstable and thus prone to abiotic hydrolysis and UV degradation (Deshpande et al., 2004). Second, it is possible for antibiotics to adsorb tightly to components in the compost, limiting their extractability and subsequent detection (Wegst-Uhrich et al., 2014). Additionally, some antibiotics readily transform, which can affect detection or give false-negative detections (Wallace and Aga, 2016; Wallace et al., 2018). Some of these nonbiological processes may help explain the results of the composting study conducted by Ray et al. (2017), where the rapid (<1 d) disappearance of cephalirin and pirlimycin was attributed to instability at high temperatures and irreversible sorption onto humic acids, respectively. Accurately extracting and measuring antibiotics, degradation products, and conjugates in composted manures is complex (Aga et al., 2016), as are the sorption dynamics of these compounds, which are dictated by pH, chemical structure, and inorganic and organic contents of the compost, among others (Wegst-Uhrich et al., 2014; Aristilde et al., 2016; Okai-kue-Woodi et al., 2018). Resolving these complexities will be essential, however, to fully understand the effect of composting conditions on antibiotic mitigation.

Effects of Antibiotics on the Composting Process

Antibiotic residues can also affect the composting process itself by inhibiting the microbial populations actively degrading the manure. Inglis et al. (2010) found that manure from cows fed AS700 (a feed supplement containing chlortetracycline and sulfamethazine) composted less consistently and did not self-heat to the same extent as antibiotic-free manure composts. Others have made similar observations and noted the effects of antibiotics on the compost microbial communities (Ray et al., 2017; Qian et al., 2018). These effects are antibiotic and concentration dependent, with tetracyclines (i.e., chlortetracycline) but not macrolides (i.e., tylosin) shown to affect the composting process (Cessna

et al., 2011). Interestingly, Hao et al. (2011) showed that antibiotic residues can also influence greenhouse gas emissions from composted manure, suggesting that the effects of antibiotics on the compost microbial community may have other unforeseen environmental consequences beyond AR.

Effect of Composting on ARB

Although antibiotic levels universally decline during the composting process (albeit to varying degrees), the effect of composting on ARB and ARG is less consistent. Composting compared with stockpiling appears to be more effective at reducing ARB, although ARB may persist in finished cow manure-based composts and facilitate the proliferation of ARB in soils when these materials are used. Walczak and Xu (2011) found that the prevalence of antibiotic-resistant *E. coli* and *Enterococcus* spp. increased in short-term cow manure stockpiles. Although Sharma et al. (2009) measured a reduction in ARB counts during windrowing, some antibiotic-resistant *E. coli* remained detectable even after 126 d. A screen of commercially available organic fertilizers also found streptomycin-resistant *E. coli* in 13% of cow manure-based composts (Miller et al., 2013). Even though antibiotic-resistant *Salmonella* were not found, the use of all but 2 of these organic fertilizers in soils resulted in growth of *E. coli* and *Salmonella*. Another study of dairy manure composts found adequate treatment of antibiotic-resistant *E. coli* and *Salmonella* but noted that the prevalence of some antibiotic-resistant *Enterobacter* spp. and multidrug-resistant *Pseudomonas* spp. increased when these composts were amended to rangeland soils in Texas (Edrington et al., 2009). These studies suggest that although medically relevant ARB were mitigated, their persistent ARG may have been transferred to commensal soil bacteria and continued to pose some risk.

Effect of Composting on ARG

Many ARG persist, with over 50 of these genes detectable in finished cattle manure composts (Qian et al., 2018). In an early study of windrowed manures from dairy cows and other domesticated ungulates, the concentration of most ARG remained stable after 182 d, regardless of manure type, the addition of bulking agents, water management, turning, or the decline in antibiotic residues observed (Storteboom et al., 2007). Sharma et al. (2009) similarly showed that most tetracycline and erythromycin ARG were stable over a 126-d study of windrow-composted cattle manure, whereas some ARG increased. In contrast, Xu et al.

(2016) found a significant (nearly 3 orders of magnitude greater) reduction in ARG levels during cattle manure composting compared with stockpiling. Other recent studies have noted significant reductions in the diversity and relative abundance of ARG and mobile genetic elements in actively managed and aerated cattle manure composts (Tien et al., 2017; Gou et al., 2018). Despite the reductions noted in these recent studies, ARG were still detectable in all finished composts analyzed.

Achieving adequate temperature regimens might be critical to the ability of a compost to more fully mitigate ARG. Cow manure composts reaching, or maintained at, thermophilic conditions were more capable of reducing ARG than composts not exceeding mesophilic conditions (Qian et al., 2016). In fact, only thermophilic conditions were able to completely mitigate some ARG (*tetC*, *tetG*, and *tetQ*) and integrons (gene associated with horizontal gene transfer, *intI1*). Xie et al. (2016) also showed that thermophilic composting of cow manure enabled ARG mitigation, lowering 16S rRNA normalized levels of tetracycline, sulfonamide, and fluoroquinolone resistance genes by nearly 2 orders of magnitude. However, some ARG (e.g., *aadA*, *aadA2*, *qacED1*, *tetL*) and mobile genetic element marker genes (e.g., *cintI1*, *intI1*, and *tnpA04*) remained. Other than research into their effects on compost stability (e.g., Kalamdhad and Kazmi, 2009), studies of commercial-scale, rotary drum systems commonly used by dairy operations in US for bedding recovery are lacking, though their control systems and thermophilic operation may be a promising treatment for ARG.

Variables other than compost management also dictate compost resistomes and their fate. These include the concentration of residual antibiotics, the co-occurrence of ARG and mobile genetic elements, and factors such as nutrient and heavy metal contents, which may relate to bacterial gene regulation and transfer (Qian et al., 2016, 2018). To provide dairy farmers with useful management strategies, both the baseline levels of ARG in manure and compost and the many confounding factors affecting the fate of ARG in composts must be resolved.

ANAEROBIC DIGESTION

Anaerobic digestion is an advanced treatment used by fewer than 200 US dairy operations, where microbial consortia under anoxic conditions degrade manure and other organics and generate digestate and biogas. Digesters can be designed for various substrates, with continuous or intermittent loading and mixing, using a range of hydraulic retention times, and operated at mesophilic or thermophilic temperatures. These differ-

ent operating conditions influence the development of the digester microbial community (Kundu et al., 2017) and consequently the abilities of digesters to mitigate AR (Youngquist et al., 2016).

Influence of Digester Operating Conditions on Antibiotic Residue Degradation

Generally, steadily operated, higher-temperature digesters are better at degrading antibiotics. Akyol et al. (2016a) found a 10% greater reduction in oxytetracycline in a 2-stage continuous-flow mixed digester compared with a less stable, single-stage system operated with the same feedstock (dairy manure) with oxytetracycline loading rates of 3 mg L⁻¹, an hydraulic retention time of 20 d, and an operating temperature of 37°C. Oxytetracycline degradation was 21 to 38% higher in a follow-up study using similar manure, where the single-stage digester was operated at 55°C (Akyol et al., 2016b). Stable operating conditions and quality feedstocks similarly improved oxytetracycline degradation in thermophilic dairy manure digesters (Türker et al., 2013). Higher antibiotic degradation rates observed in thermophilic digester systems were attributed to optimized microbial kinetics and the facilitation of pyrolysis reactions by higher reaction temperatures (Varel et al., 2012; Lin et al., 2017).

Antibiotic Degradation in Digesters Is Antibiotic Dependent

In cattle manure-based digesters, antibiotic degradation products and the formation of recalcitrant, and sometimes bioactive, epimeric degradation depends on the antibiotic and its concentration. For example, in laboratory bottle tests with dairy manure, Mitchell et al. (2013) observed 3 distinct degradation patterns depending on the class of the antibiotic: (1) no degradation regardless of concentration (e.g., sulfamethazine), (2) concentration-dependent degradation rates (e.g., ampicillin), and (3) the formation of persistent degradation products not generated when initial concentrations were lower (e.g., florfenicol). Degradation rates can also differ for antibiotics within the same class due to variances in antibiotic chemical stability, biodegradability, solubility, and sorption properties (Mitchell et al., 2013). For example, even though sulfadiazine and sulfamethazine differ by only 2 methyl groups, these sulfonamides had degradation rates differing by as much as 30% in cow manure digester trials (Spielmeyer et al., 2015). Similar differences have been observed in full-scale dairy manure-based digesters for tetracycline antibiotics (Wallace et al., 2018) and non-medically

important ionophores (Varel et al., 2012; Arikan et al., 2018).

Antibiotic Residuals in Manure Can Affect Digester Performance

It has also long been recognized that antibiotic residues in cow manure can affect the anaerobic digestion process (Gamal-El-Din, 1986). For example, oxytetracycline has been shown to impair essential digester microbial groups, including methanogens (Akyol et al., 2016a,b; Türker et al., 2016) and can significantly reduce biogas yields (Spielmeyer et al., 2014). The effect of antibiotics on biogas production is not always consistent (Spielmeyer et al., 2015) and, in some cases, initial inhibition in biogas production was overcome in time (Ince et al., 2013; Mitchell et al., 2013). Digester studies have also shown that higher oxytetracycline concentrations can have more significant effects on volatile fatty acid generation (Akyol et al., 2014) and subsequent biogas and methane CH₄ yields (Akyol et al., 2016b), but under stable reactor conditions, methanogens were more resilient to these effects (Akyol et al., 2016a). Other studies confirm that different levels of oxytetracycline can variably affect biogas production in both cattle manure-based digesters (Arikan et al., 2006; Arikan, 2008) and expanded granular sludge bed reactors treating dairy manure (Londoño et al., 2012).

The effect of β -lactams and other antibiotics is less clear. In one study, cefazolin had no effect on CH₄ production in a cow manure-based digester, either alone or combined with oxytetracycline, regardless of concentration (Beneragama et al., 2013b); in another study, cefazolin impaired biogas production at concentrations >10 mg L⁻¹ (Kitazono et al., 2015). Other antibiotics, including ionophores, can also affect digester performance, although the concentrations tested in these studies were much greater than what would be experienced under actual on-farm operating conditions (Mitchell et al., 2013; Arikan et al., 2018). In general, most of the anaerobic digestion studies dealing with cattle manure and antibiotics have not adequately represented real-world conditions or have focused on one antibiotic residue, namely oxytetracycline.

The challenges associated with comprehensive, on-farm digester research are significant. Although mimicking on-farm dynamics is also not easy, future studies should try to use more representative antibiotic residues mixtures, concentrations, and operating conditions. Only with such studies can the combined effects of antibiotics, system performance, and digester microbial communities be assessed and optimized for impacts on AR residue mitigation.

ARB in Anaerobic Digesters

Although ARB are usually not prevalent in the effluents of dairy manure-based digesters, they can be detected (Lateef et al., 2012; Resende et al., 2014b). As with antibiotic residues, thermophilic conditions appear to be better at reducing pathogens (Varel et al., 2012) and ARB (Beneragama et al., 2012, 2013a). Interestingly, ARB populations will sometimes rebound in anaerobic digesters following an initial decline. In a mesophilic digester treating manure slurry and waste milk, >99% of ARB were removed after 20 d, but around 40% of the initial count of ARB were once again detectable by d 34 (Beneragama et al., 2013c). In another study, Beneragama et al. (2013a) observed that despite an initial decrease in ARB resistant to ceftiofur, counts eventually rose to near starting levels. These patterns are likely explained by differential death and survival of ARB and horizontal transfer of ARG to digester bacterial groups (Miller et al., 2016; Sun et al., 2016), though the dynamics of antibiotic resistance development, proliferation, and maintenance are complex and have not been extensively studied in dairy manure-based digesters.

Fate of ARG in Anaerobic Digesters

Complex interactions between operating conditions, resident microbial communities, and the selective pressures of antibiotic residues dictate the fate of ARG in anaerobic digesters. Generally, digesters modestly reduce ARG, but this appears to depend on the ARG and digester operating temperature. Tien et al. (2017) found 11 ARG targets to be lower in digestate than in raw manure, but this was only significant for 2 ARG. Wallace et al. (2018) observed a significant decrease in sulfonamide resistance genes during mesophilic digestion of cow manure, but no change in tetracycline resistance genes, which was attributed to the persistent selective pressure of tetracycline antibiotics in the digestate. Sun et al. (2016) noted that although sulfonamide resistance genes and other ARG declined regardless of digester operating temperature, tetracycline resistance genes only decreased when the operating temperature was in the thermophilic range. With a long hydraulic retention time (60 d), ambient temperature (14 to 34°C), cow manure-based anaerobic digester in Brazil, ARG had a temperature-dependent fate, with genes encoding resistance to macrolides, aminoglycosides, and β -lactams being reduced to a greater extent during the summer when operating temperatures were significantly higher (Resende et al., 2014a). These temperature responses are likely related to their associated digester microbial

community shifts and associated propensity of some ARG to horizontally transfer from manure bacteria to populations inside the digester (Sun et al., 2016). More work is needed in this area to understand the fate of ARG under varying temperature and digester operating conditions so that these systems may be optimally managed to mitigate ARG.

LONG-TERM MANURE STORAGE AND ANAEROBIC LAGOONS

Few studies have looked at the effect on dairy manure-associated AR in ambient-temperature, long-term storage (containments not designed for treatment) and anaerobic lagoons (containments designed to anaerobically treat manure slurries). With lagoon systems, most research has focused on swine manure slurries where, like anaerobic digesters, persistent antibiotics can sometimes affect treatment performance (Loftin et al., 2005). One bench-scale study of lagoons treating dairy manure slurry focused on ARG and noted that higher incubation temperatures were required for appreciable mitigation of ARG, with ambient temperature treatments being less capable of mitigation (Pei et al., 2007). Studies of dairy manure long-term storages in Europe have shown that some ARG may increase during storage, and others may decline, depending on the farm (Ruuskanen et al., 2016) and occurrence of horizontal gene transfer (Baker et al., 2016). Our investigation of dairy manure long-term storages and lagoons at 11 northeastern US dairy operations also found persistent ARG, primarily β -lactam and tetracycline resistance genes, as well as tetracycline residues (Hurst et al., 2019). Not surprisingly with the large volumes of these systems and limitations in collecting representative samples, significant variability was noted, which creates challenges with data interpretation. Additional research is particularly needed to understand AR in long-term storages, as they are a critical component of manure management systems on a growing number of modern dairy operations and the final opportunity to treat manure before land application.

FIELD TREATMENT SYSTEMS

Although several studies have looked at the impact of manure applications on soils and crops, few have considered field treatment systems. Chu et al. (2010) was one of the first and highlighted the potential for natural buffering of veterinary antibiotic residues in agroforestry strips, grass buffers, and even cropland soil. Woodchip denitrifying bioreactors designed to mitigate nitrogen runoff may also retain 70 to 90% of

some manure-borne antibiotics (e.g., sulfamethazine), as well as the herbicide atrazine (Ilhan et al., 2011). Additionally, evidence indicates that biochars made from agricultural and forestry residues may be excellent candidates for sequestering antibiotic residues in soil and manures (Mitchell et al., 2015a). An opportunity exists to investigate these and similar field treatments, which may develop into best management practices that help dairy operators better manage dairy manure-associated antibiotic residues in the field.

There is a need to understand the natural AR background in field systems to properly assess the impact of manure and study the efficacy of field treatments. Antibiosis is an ancient and naturally occurring process common in soils (Martinez, 2012; Cytryn, 2013; Nazaret and Aminov, 2014). Many variables influence levels of ARB, their antibiotics, and ARG found in soils today (Bueno et al., 2018), including the type of soil (Chen et al., 2018), organic amendments (Rahube et al., 2014; Kyselková et al., 2015b; Sandberg and LaPara, 2016), inorganic fertilizers (Nölvak et al., 2016), organopollutants (Sun et al., 2015), exposure history (Udikovic-Kolic et al., 2014; Topp et al., 2016), groundwater chemistry (Chen et al., 2015b), and presence of microbial biofilms (Winkworth and Lear, 2014). These altered soil levels may be brief (Winkworth et al., 2015) or long lasting (Nölvak et al., 2016) and will depend on preferential flow paths (Bradford et al., 2017), sorption processes (Pan and Chu, 2016, 2017b), and other complex transport mechanisms (Jechalke et al., 2014; Nesme and Simonet, 2015; Pollard and Morra, 2018). To address the issue of determining the natural background levels of AR in agroecosystems, Rothrock et al. (2016) noted the need for normalization within studies and between studies, the standardization of analytical approaches, identification of drug residue, ARB and ARG targets, and subsequently, a need for surveillance of these targets.

SUMMARY AND RESEARCH NEEDS

The anthropogenic and environmental dimensions of AR in dairy manures are complex and influenced by many factors. Larsson et al. (2018) considers 4 major knowledge gaps to the environmental dimensions of AR: (1) the relative contributions of AR from different sources, (2) the role of the environment in the spread of AR, (3) the risk of human exposure to AR in the environment, and (4) the development of mitigation strategies; all are applicable to dairy operations. Resolving levels of antibiotic residues, ARB, and ARG in a broader sampling of dairy manures is primary. These data are critically needed to assess baseline levels and

how they change over time in response to farm management and antibiotic usage. Similarly, it is necessary to determine background levels of AR in the dairy agroecosystem so that the actual impact of treated and untreated manures on field soils, crops, and the environment can be assessed. Testing of currently used manure management systems and emerging advanced treatments for their ability to degrade antibiotic residues, control ARB and ARG, and mitigate the spread of AR is also warranted. The concentration data from field research must be normalized on a mass basis, because concentrations can be misleading when assessing manure treatment processes due to their complex effect on, and the variability of, manure composition. Supportive experimental work that models on-farm conditions is necessary to complement this applied research. Collectively, these efforts will build an understanding of on-farm exposure routes and risks and the environmental pathways by which AR may spread from or onto a farm, as well as identify farm practices that may limit its spread. Most imperative is that stakeholders help advise these research efforts, so that scientific findings are more easily translated into practical on-farm management decisions and best equip the dairy industry with the resources and tools needed to respond to the global AR crisis.

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